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Supporting Online Material for

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Published 8 July 2010 on *Science* Express
DOI: 10.1126/science.1192819

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MATERIALS AND METHODS

Expression and purification of HIV-1 gp120 proteins

The codon-optimized plasmid pVRC8400-HIV-1 Clade A/E 93TH057 ΔV123 contains gp120 residues 44-492 with specific deletions at the V1/V2 and V3 regions. The expression construct was made by inserting mouse interleukin-2 (IL-2) leader sequence (MYSMQLASCVTLTLVLLVN) followed by the modified gp120 sequence between the 5' XbaI and 3' BamHI sites. In the modified gp120 sequence, N-term residues 31-43 of wild type Clade A/E 93TH057 gp120 were trimmed, amino acids sequences ^{124} PLCVTLHCT TAKLTNVTNITNVPNIGNITDEVRNCSFNMTTEIRDKKQKVHALFYKLDIVQIEDKN DSSKYRLINCNT ^{198} of the V1/V2 loop and ^{302}NMRTSMRIGP GQVFYRTGSIT^{323} of the V3 loop were replaced with GG and GGSGSG linkers, respectively.

YU2 Δβ4 core gp120 was constructed as described (S*1*) with YU2 gp120 sequence by replacing the 9 residues of β3-β5 loop with a Gly-Gly linker. This deletion weakens the binding of CD4 and prohibits formation of the antibody 17b epitope.

The 93TH057, YU2Δβ4 core gp120 and HXBc2 Ds12F123 core gp120 (S*2*) were expressed and purified as described previously (S*3*). Briefly 1L of HEK 293 GnTi$^-$ or 293 FreeStyle cells were transiently transfected with the mixture of 500 μg of gp120 DNA plasmid and 1 ml of 293fectin (Invitrogen). The transfected cells were incubated in FreeStyle 293 expression medium (Invitrogen) supplemented with 3% Cell Boost (HyClone) and 2 mM Butyrate (SIGMA) for suspension culture at 8% CO_2, 37.0 °C and 125 rpm for five days after transfection. The supernatants for 93TH057 and HXBc2 Ds12F123 core gp120 were harvested and proteins purified with a protein A-immobilized 17b antibody affinity column. The YU2Δβ4 core gp120 was purified with a protein A-immobilized F105 antibody affinity column. The gp120 proteins were eluted with IgG elution buffer (Pierce) and immediately adjusted to pH 7.5.

Production of VRC01 IgG and antigen-binding fragment

The VRC01 IgG was expressed and purified as described in the companion paper (S*4*). Briefly, heavy and light chain plasmids were transfected into 293F cells using 293Fectin (Invitrogen). The supernatant was harvested 5 days after transfection, filtered through 0.45 µm filter, and followed by purification using immobilized protein A or protein G columns. To produce antigen-binding fragments (Fab), VRC01 IgG was incubated at 37 ^{o}C with protease Lys-C (Roche) at a ratio IgG:LysC=4000:1 (w/w) in 10 mM EDTA, 100 mM Tris/Cl^-, pH 8.5 for about 12 hours. Uncleaved IgG and the constant fragment (Fc) were removed by passing the digestion mixture through a Protein A affinity column; the flow-through containing VRC01 Fab was concentrated and loaded onto size-exclusion column (Superdex S200) for further purification.

Deglycosylation, complex formation and crystallization of the gp120:VRC01 complexes

Deglycosylation of HIV-1 gp120 was performed in a reaction solution containing 1-5 mg/ml gp120, 350 mM NaCl, 100 mM Na Acetate, pH 5.9 , 1x EDTA-free protease inhibitor (Roche) and endoglycosidase H (30 units/µg of gp120). After mixing all components and adjusting pH to 5.9, the solution was incubated at 37^{o}C and the deglycosylation process was monitored by SDS-PAGE until completion.

gp120:VRC01 complexes were made following procedures that were previously described (S3). Briefly, VRC01 Fab in 20% molar excess was combined with deglycosylated gp120 and the gp120:VRC01 mixture was passed through a concanavalin A column to remove gp120 with uncleaved N-linked glycans. The complex was then purified by size exclusion chromatography (Hiload 26/60 Superdex S200 prep grade, GE Healthcare) and concentrated to ~10 mg/ml in 0.35 M NaCl, 2.5 mM Tris pH 7.0, 0.02% NaN_3 for crystallization screening experiments. To achieve better chances of crystallization hits, two gp120 variants, clade B HxBc2 core Ds12F123 and clade A/E 93TH057, were used to make complexes with the VRC01 Fab.

Commercially available screens, Hampton Crystal Screen (Hampton Research), Precipitant Synergy Screen (Emerald BioSystems), and Wizard Screen (Emerald BioSystems), were used for initial crystallization trials of the gp120:VRC01 complexes. Vapor-diffusion sitting drops were set up robotically by mixing 0.1 μl of protein with an equal volume of precipitant solutions (Honeybee, DigiLab). Droplets were allowed to equilibrate at 20º C and imaged at scheduled times with RockImager (Formulatrix.). Multiple crystal hits were obtained from both HXBc2:VRC01 and 93TH057:VRC01 complexes. Those hits were optimized manually using the hanging drop vapor-diffusion method. Crystals of the HXBc2:VRC01 complex were obtained in 1.0 M NaCitrate, 100mM NaCacodylate, pH 6.5. For the 93TH057:VRC01 complex, the best condition to obtain diffraction-quality crystals was 10% PEG 8000, 100 mM Tris/Cl^-, pH 8.5 with 3 % glucose as additive.

X-ray data collection, structure determination and refinement for the gp120:VRC01 complex

The diffraction of gp120:VRC01 crystals were tested under cryogenic conditions. To search for the best cryo-protectant, protecting effects of six commonly used cryo-protectants, 30% glycerol, 30% ethylene glycol, 15% 2R,3R-butanediol, 40% trihalose, 40% sucrose and 40% glucose, were assessed. Crystals were transferred into solutions which were composed of crystallization reservoir solution with 50% higher concentration of precipitant(s) and each individual cryo-protectant or mixture of cryo-protectants, immediately flash frozen in liquid nitrogen with a cryo-loop (Hampton Research) and mounted under cryo condition (100K^{o}) for data collection. X-ray data were collected at beam-line ID-22 (SER-CAT) at the Advanced Photon Source, Argonne National Laboratory, with 0.82656 Å radiation, processed and reduced with HKL2000 (S5). None of the HXBc2:VRC01 crystals diffracted beyond 4 Å resolution and they were not used for data collection. A 2.9 Å data set for the 93TH057:VRC01 crystals was collected using a cryoprotectant solutions containing 15% PEG8000, 100 mM Tris/Cl^{-}, pH 8.5 and 20% glucose and 7.5 % 2R,3R-butanediol as cryoprotectants. I/σ ratio was 1.2 at the 2.9 Å shell with 68% completeness.

The crystal structure of the 93TH057:VRC01 complex was solved by molecular replacement with Phaser (S6) in the CCP4 Program Suite (S7). This crystal belonged to a space group P21 with cell dimensions a=108.6, b=98.3, c=205.3, β=99.7 and contained four molecules per asymmetric unit. The structure of 93TH057 gp120 with β20/β21 region trimmed (PDB #3M4M) was used as an initial model to place the gp120 in the complex. Phaser was able to give a solution with three gp120s initially (RFZ=4.3 TFZ=4.7 PAK=0 LLG=69

RFZ=3.5 TFZ=11.8 PAK=0 LLG=234 RFZ=3.7 TFZ=17.5 PAK=0 LLG=485 LLG=1280). With those three gp120s fixed, the CDR-loop-trimmed variable domain (Fv) of antibody b13 (PDB ID 3IDX) (S*3*) was used to locate the Fv portion of VRC01 in the complex (RFZ=3.7 TFZ=11.7 PAK=0 LLG=539 RFZ=3.9 TFZ=5.0 PAK=0 LLG=389 RFZ=3.9 TFZ=4.7 PAK=0 LLG=136 LLG=428). Visual inspection of the generated gp120:Fv solutions identified one of gp120s complexed with a symmetry-operated Fv. This new gp120:Fv complex was used as model to perform a new round of molecular replacement, one complex at a time, until all four gp120:Fv complexes were found (Round 1: RFZ=6.6 TFZ=8.9 PAK=0 LLG=72 LLG=243, Round 2: RFZ=5.9 TFZ=16.0 PAK=0 LLG=78 LLG=623, Round 3: RFZ=8.4 TFZ=25.3 PAK=0 LLG=739 LLG=1866, Round 4: RFZ=5.8 TFZ=22.8 PAK=0 LLG=990 LLG=2608). The constant domain of Fab b13 was then used to place one of the VRC01 constant domains with all four previous solutions fixed (RFZ=4.7 TFZ=21.5 PAK=1 LLG=1357 LLG=3313). The newly found constant domain and the gp120:Fv formed a complete gp120:VRC01 complex and the other three molecules were generated by superposing this complex with other three gp120s in the asymmetric unit.

Further refinement was carried out with PHENIX (S*8*). Starting with torsion-angle simulated annealing with slow cooling, iterative manual model building was carried out on Xtalview (S*9*) and COOT (S*10*) with maps generated from combinations of standard positional, individual *B*-factor, TLS refinement algorithms and non-crystallographic symmetry (NCS) restraints. Ordered solvents were added during each macro cycle. Throughout the refinement processes, a cross validation (R_{free}) test set consisting of 5% of the data was used. Structure validations were performed periodically during the model building/refinement process with MolProbity (S*11*) and pdb-care (S*12*). Even though the reported data at the highest shell of 2.9 Å only has I/σ ratio of 1.2, reflections up to 2.7 Å resolution (I/σ >1.0, ~30% completeness) were included and used during the refinement. X-ray crystallographic data and refinement statistics are summarized in Table S1.

Surface plasmon resonance (SPR)

The binding kinetics of HIV-1 gp120 with different ligands were performed on Biacore 3000 or Biacore T-100 (GE Healthcare) at 20°C with buffer HBS-EP+ (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, and 0.05% surfactant P-20). To assess VRC01 recognition of gp120 in the CD4-bound and non-CD4-bound conformation, gp120 molecules (YU2Δβ4 core and HXBc2 Ds12F123 core) were immobilized onto a CM5 chip to 250-500 response units (RUs) with standard amine coupling; Fab antibodies, CD4 or CD4-IgαTP at 2-fold increasing concentrations were injected over the gp120 channels at a flow rate of 30 μl/min for 3 minutes and allowed to dissociate for another 5-10 minutes before regeneration with two 25 μl injections of 4.5 M $MgCl_2$ at a flow rate of 50 μl/ml.

To test the effects of mutations on VRC01, IgG variants were captured with a mouse anti-human IgG Fc antibody supplied in the "human antibody capture kit" (GE Healthcare) to a surface density about 300 RUs. gp120 series with 2-fold increasing concentrations were passed over the captured IgG flow channels for 3 minutes and allowed to dissociate for another 5-10 minutes at a flow rate of 30 μl/min. The sensor chip was regenerated after each experiment using two 25 μl injections of 4.5 M $MgCl_2$ at a flow rate of 50 μl/ml.

Sensorgrams were corrected with appropriate blank references and fit globally with Biacore Evaluation software using a 1:1 Langmuir model of binding. Sensorgrams of IgG b12 binding to gp120 could not be fitted with 1:1 Langmuir model and were analyzed with a two-state binding model; such treatment should not affect the primary on-rates nor overall K_Ds reported here.

Mutagenesis and creation of VRC01 genomic revertants

Single- 4-, 7- , 12-revertant mutations to germline residues of VRC01 as well as the heavy chain C32SC98A mutations, light chain insertions with Ala-Ala or Ser-Tyr after position 30 were listed in Table S12 and the mutagenesis were carried out using Quikchange kit (Stratagene) according to manufacturer's protocol.

V-gene revertants for VRC01 were constructed as follows: For heavy chain V-gene revertant (gH), VRC01 heavy chain V-gene region was reverted to its germline precursor IGHV 1-02*02. For light chain V-gene revertant (gL), VRC01 light chain V-gene region was reverted to its germline precursor IGKV 3-11*01. The modified heavy and light chain genes were synthesized by GeneArt (Regensburg, Germany), and cloned into a mammalian CMV/R vector for expression. All the VRC01 variants were expressed with the same protocol as wild type VRC01 IgG.

Neutralization assays

Neutralization assays of viruses by VRC01 and its variants

HIV-1 Env-pseudoviruses were prepared by transfecting 293T cells (6 x 10^6 cells in 50 ml growth medium in a T-175 culture flask) with 10 µg of *rev*/*env* expression plasmid and 30 µg of an *env*-deficient HIV-1 backbone vector (pSG3ΔEnvelope), using Fugene 6 transfection reagents (Invitrogen). Pseudovirus-containing culture supernatants were harvested two days after transfection, filtered (0.45 µm), and stored at –80°C or in the vapor phase of liquid nitrogen. Neutralization was measured using HIV-1 Env-pseudoviruses to infect TZM-bl cells as described previously (S*13-14*). Briefly, 40 µl of virus was incubated for 30 min at 37ºC with 10 µl of serial diluted test antibody in duplicate wells of a 96-well flat bottom culture plate. To keep assay conditions constant, sham media was used in place of antibody in specified control wells. The virus input was set at a multiplicity of infection of approximately 0.01, which generally results in 100,000 to 400,000 relative light units (RLU) in a luciferase assay (Bright Glo, Promega, Madison, WI). The antibody concentrations were defined at the point of incubation with virus supernatant. Neutralization curves were fit by nonlinear regression using a 5-parameter hill slope equation as previously described (S*15*). The 50% inhibitory concentrations (IC_{50}) were reported as the antibody concentrations required to inhibit infection by 50%.

Neutralization assay of viruses with altered sampling of the CD4-bound state by VRC01 and CD4

The assay was performed as previously described (S*16*). Recombinant HIV-1 expressing the firefly luciferase gene was produced by calcium phosphate transfection of 293T cells with the molecular clone pNL4.3 (Env-) Luc and the pSVIIIenv plasmid expressing the wild-type or mutant HIV-1_{YU2} envelope glycoproteins at a weight ratio of 2:1. Two days after transfection, the cell supernatants were harvested. The reverse transcriptase activities of all virus preparations were measured, as described previously (S*17*). Each virus preparation was frozen and stored in aliquots at -80 °C until use. Luciferase-expressing viruses bearing either wild-type or mutant envelope glycoproteins were incubated for 1 hour at 37°C with serial dilutions of sCD4 or VRC01 IgG in a total volume of 200 μl. The recombinant viruses were then incubated with Cf2Th-CD4/CCR5 cells; luciferase activity in the cells was measured two days later.

ELISA assay

Clade A/E 93TH057 and clade B HXBc2 core Ds12F123 gp120 in PBS (pH 7.4) at 2μg/ml were used to coat plates for 2 hours at room temperature (RT). The plates were washed five times with 0.05% Tween 20 in PBS (PBS-T), blocked with 300 μl per well of block buffer (5% skim milk and 2% bovine albumin in PBS-T) for 1 hour at RT. 100 μl of each monoclonal antibodies 5-fold serially diluted in block buffer were added and incubated for 1 hour at RT. Horseradish peroxidase (HRP)-conjugated goat anti-human IgG (H+L) antibody (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) at 1:5,000 was added for 1 hour at RT. The plates were washed five times with PBS-T and then developed using 3,3´,5,5´-tetramethylbenzidine (TMB) (Kirkegaard & Perry Laboratories) at RT for 10 min. The reaction was stopped by the addition of 100μl 1 N H_2SO_4 to each well. The readout was measured at a wavelength of 450nm. All samples were performed in triplicate.

Analysis of the commonality of VRC01 features

Structural dataset for analysis of antibody affinity maturation

Initially, the IMGT/3Dstructure-DB (S*18*) was searched for structures of human antibody-protein and antibody-peptide complexes of at most 3.5Å resolution. This resulted in a set of 54 antibody-protein and 66 antibody-peptide structures (the 2wuc complex was found in both the protein and peptide databases). To complement the structure query, the RSCB PDB (S*19*) was searched for "human fab complex" and structure resolution of at most 3.5Å, resulting in a set of 290 structure hits; 211 of these had not been identified as part of the IMGT/3Dstructure-DB search. All unique pdbs from the IMGT/3Dstructure-DB database and the PDB search were manually inspected. Additionally, two structures (2b4c and 3hi1, see Table S11) not found in either search were also inspected.

To be included in the affinity maturation analysis, pdbs had to possess the following properties: antibody-protein or antibody-peptide complex, IgG antibody of human origin, natural affinity maturation, and antigen not artificially modified for improved binding. In the cases where multiple complexes of the same antibody were identified, only one such complex was used for the analysis. As a result, only 26 of the complexes, shown in Table S10, were retained for the antibody affinity maturation analysis. The list of discarded pdbs, along with the specific reasons for discarding, is shown in Table S11.

For each of the selected 26 andibody complexes, the number of antibody contact residues that were mutated from germline was computed for the antibody V-segments. Contact residues were identified using PISA (S*20*). Antibody germline genes were identified with IgBLAST (http://www.ncbi.nlm.nih.gov/igblast/) using antibody protein sequences for the search; insertions/deletions were not counted toward the number of mutations from germline. A summary of the number of contact residues, V-segment mutated residues, and mutated contact residues for the 26 antibodies are shown in Table S10.

Analysis of cys residues, residue deletions, and glycan additions

A dataset of human HIV-1 antibody heavy and light chain sequences was obtained from (S*21*). The final curated version of that dataset that excluded non-specific gp140 binders as well as sequences with non-fully-resolved variable regions, included 147 heavy and 147 light chain sequences (Appendix).

Sequence alignment to germline was performed using IMGT/V-QUEST (S*22*). The number of glycans was computed for the V-D-J heavy and V-J light regions. The number of Cys residues was computed for the V-D-J heavy regions only. The number of residue deletions was computed for the V-segments as compared to the corresponding germline; a deletion of multiple consecutive amino acids was counted as a single deletion.

Numbering of amino acid residues in antibody

We follow the Kabat (S*23*) nomenclature for amino acid sequences in antibodies.

Protein structure analysis and graphical representations

GRASP (S*24*) and APBS (S*25*) were used in calculations of molecular surfaces, volumes, and electrostatic potentials. PISA (S*20*) was used to perform protein-protein interfaces analysis. CCP4 (S*10*) was used for structural alignments. All graphical representation with protein crystal structures were made with Pymol (S*26*).

SUPPLEMENTARY FIGURES AND TABLES

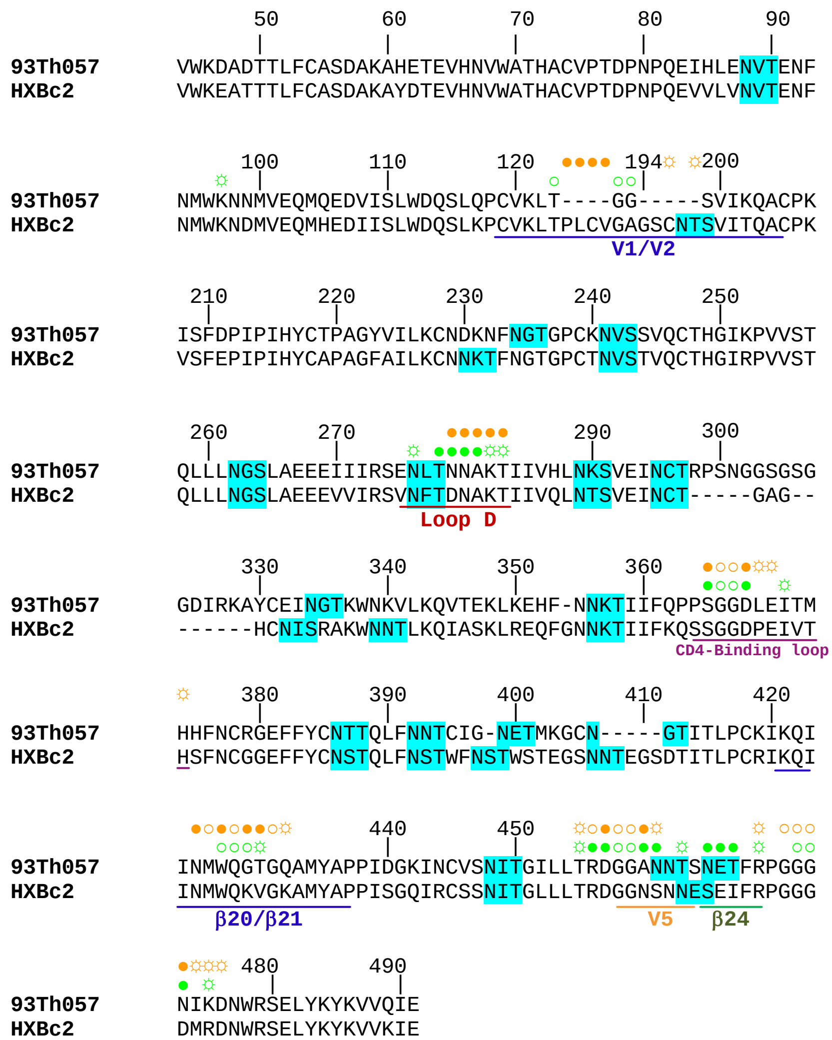

Figure S1. gp120 sequence alignment and residue-by-residue contacts with CD4 and VRC01.

Both wild type clade B HXBc2 and clade A/E 93TH057 core gp120 sequences are displayed with HXBc2 numbering convention. The 93TH057 construct has shorter V1/V2 stem and has a new V3 stem as described in Material and Methods. gp120 contacts as defined with the program PISA (S*20*) for the CD4 and VRC01 complexes are indicated in orange and green, respectively, with open circles (○)denoting gp120 main-chain-only contacts, open circles with rays (☼)denoting gp120 side-chain-only contacts, and filled circles (●) denoting both main-chain and side-chain contacts. The major structure elements of gp120 that involved in ligand binding were underlined. Potential glycosylation sites on gp120 with signature sequence NXT/S are highlighted in cyan, however, not all sites are observed in the crystal structure. VRC01 has remarkably less interactions with the conformationally variable V1/V2 and β20/β21 regions and more interactions at the loop D and V5 areas.

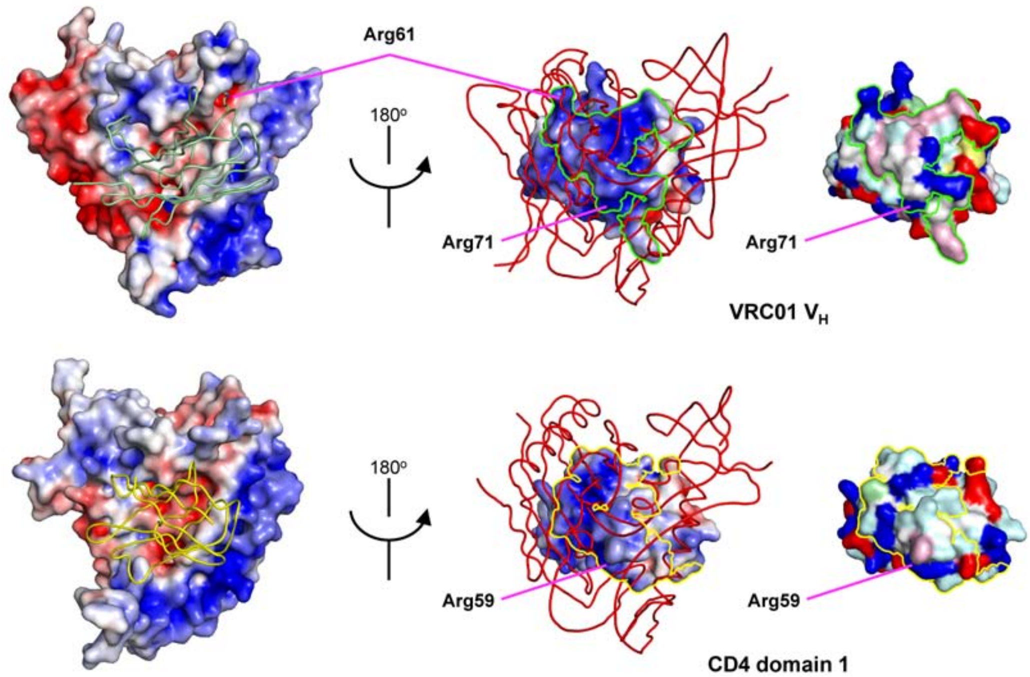

Figure S2. Electrostatic surfaces and maps of residues types.
Electrostatic surfaces for the VRC01 and CD-bound gp120s are shown in the left panels with heavy chain variable domain (V_H) of VRC01 (palegreen, upper row) and domain 1 (D1) of CD4 (yellow, lower row). Both of VRC01 and CD4 bind to overall negatively charged surfaces on gp120. The flip sides of the complexes showing the electrostatic surfaces of V_H and D1 are presented in the middle panels with gp120 (red tubes) in the foreground. Molecular surfaces of V_H and D1 colored by residue types were shown to the right. Green and yellow trace-lines define gp120 footprints on V_H and CD4, respectively. The gp120 interfaces on V_H of VRC01 and D1 of CD4 are mostly positively charged to complement the negatively charged gp120 surfaces. Certain residues, such as Arg71 in VRC01 and Arg59 in CD4, are conserved, the unique VRC01 Arg61 that penetrating the cavity formed by V5 and β24 is also shown. The electrostatic potential was calculated with APBS (S*25*) and visualized with Pymol (S*26*) using blue and red representing positive and negative charges, respectively. Color representation of residue types are white for hydrophobic, yellow for semi-polar, cyan for polar, blue for positive, red for negative and pink for aromatic.

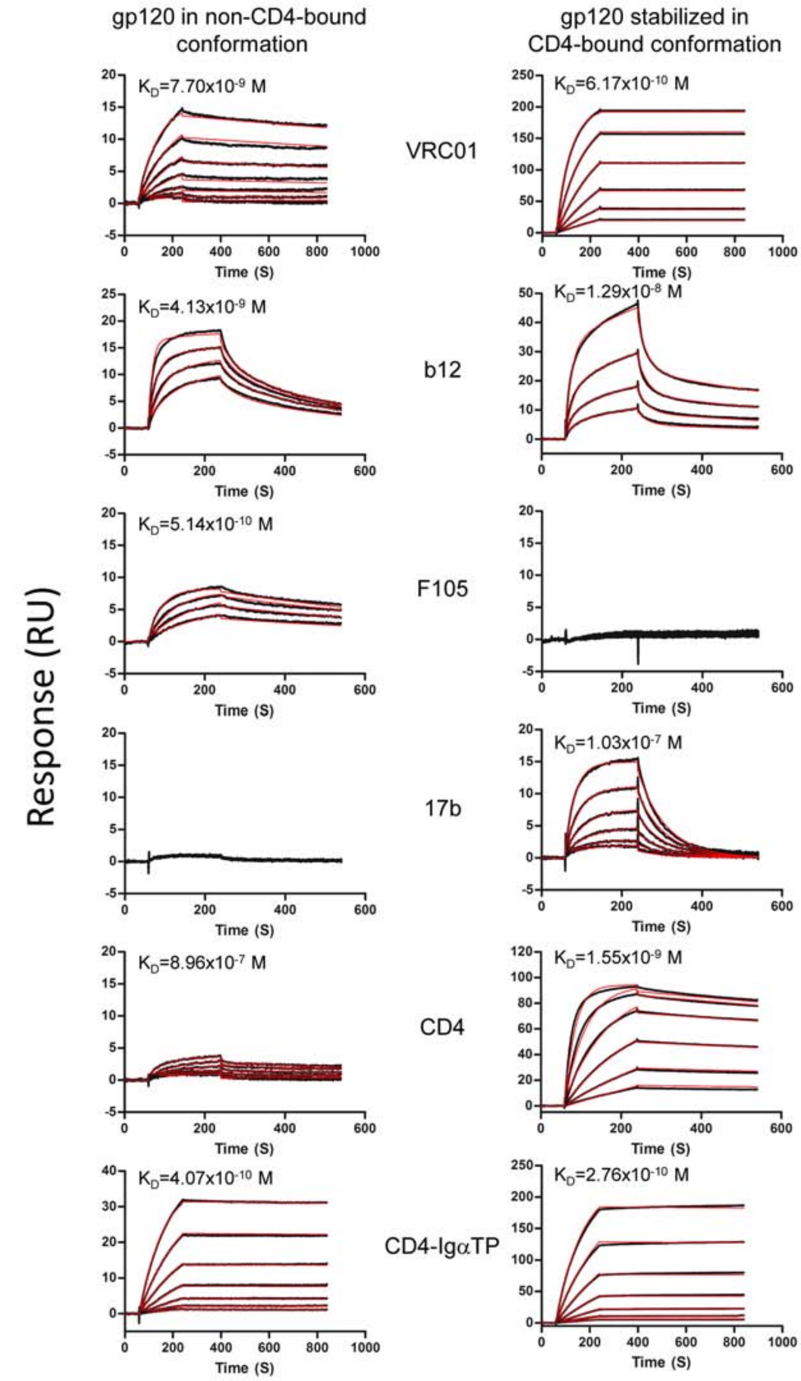

Figure S3. Binding of VRC01 to gp120 stabilized in CD4-bound and non-CD4-bound conformations.

Both gp120 in non-CD4-bound conformation (YU2 Δβ4) and gp120 stabilized in CD4-bound conformation (HXBc2 core Ds12F123) were immobilized on a CM-5 chip. Fabs of CD4-binding site antibodies VRC01, b12 and F105 and CD4-induced antibody 17b, two-domain CD4 and CD4-IgαTP at various concentrations were injected over the chip channels as described in Material and Methods. Sensorgrams are shown in black and the fitted curves in red.

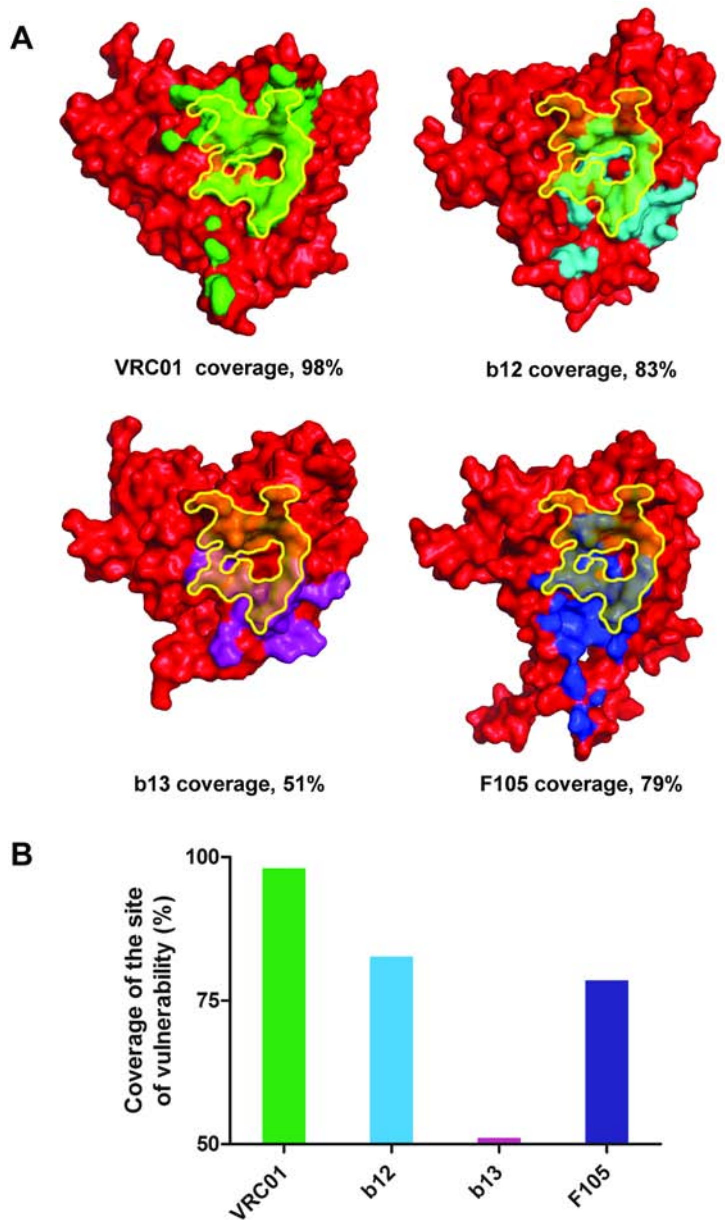

Figure S4. Comparison of coverage of the site of vulnerability by different CD4-binding site antibodies

The site of vulnerability is the contact site for receptor CD4 on the outer domain of gp120. CD4-binding-site-directed antibodies target this general area, however, most of them do not neutralize potently. Panel A: When the site of vulnerability (semi-transparent yellow with solid yellow borderline) is superimposed over the antibody epitopes on gp120 surfaces (red), the degrees of overlapping differ. VRC01(green) hits the "bull's-eye" while b12 (cyan), b13 (purple) and F105 (blue) miss portions of the target with epitope straying away to other conformationally variable areas on gp120. Panel B: Coverage of the site of vulnerability by epitopes of CD4-binding-site-directed antibodies were compared, VRC01 achieves almost full coverage (98%) while others, such as b13, F105 and b12, manage to get 50% to 83% overlapping.

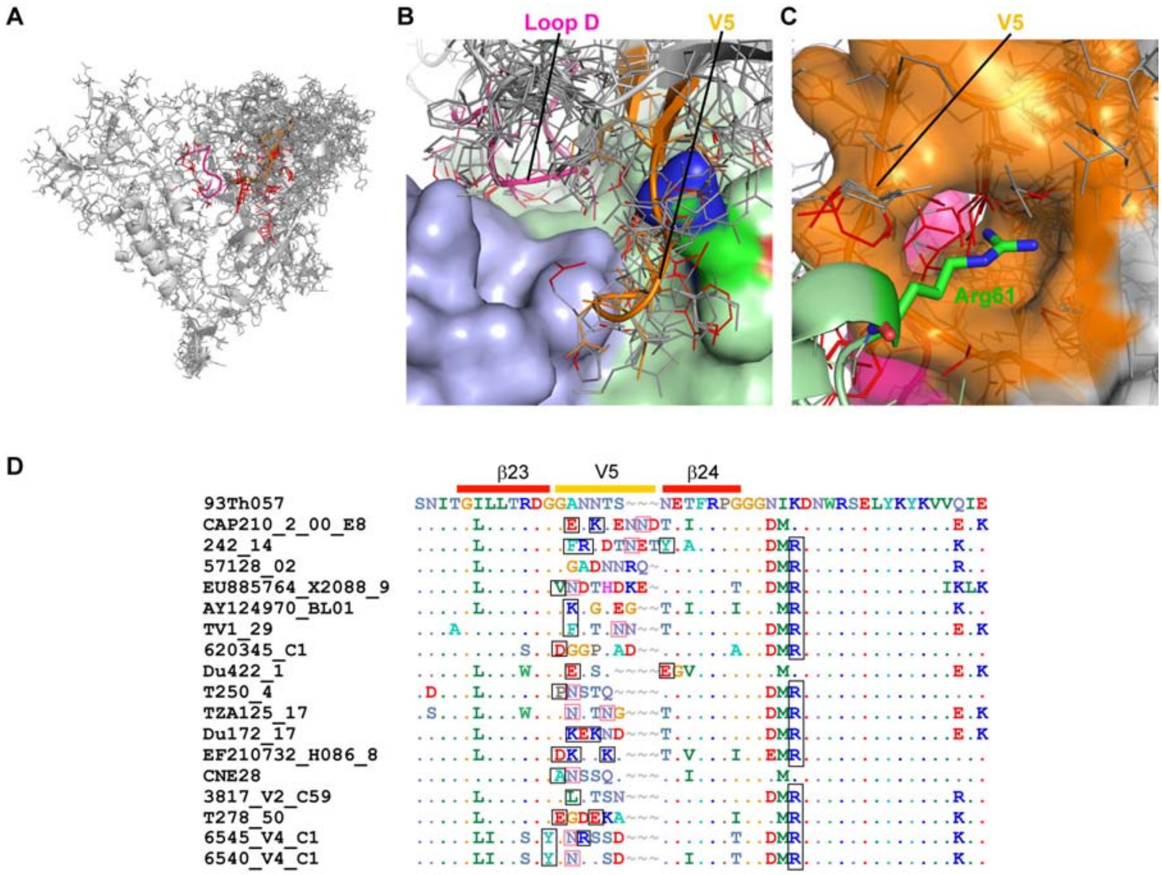

Figure S5. Mechanism of natural resistance to VRC01

(A) Sequence threading of the 17 HIV-1 isolates that resist neutralization by VRC01. Spots that are closer than 2.5 Å to VRC01 are colored red. These spots are clustered at the loop D and V5 region on HIV-1gp120. (B) Close-up of threaded, resistant isolates are shown along with the molecular surface of VRC01, colored light blue for the light chain and green for the heavy chain. Clashes predicted to interfere with VRC01-gp120 interactions are highlighted in red. (C) VRC01 heavy chain Arg61 penetrating the gp120 cavity formed by V5 and β24 (orange). Some resistant isolates have bulky residues pointing into the cavity which interfere with $\mathrm{Arg61}_{\mathrm{VRC01}}$ without affecting CD4 binding. (D) Sequence alignment of VRC01-resistant isolates at the V5 region. a black boxes highlight bulky residues that may interfere with binding of VRC01 and are different from the 93TH057 sequence. Different N-linked glycosylation patterns are also marked with red boxes.

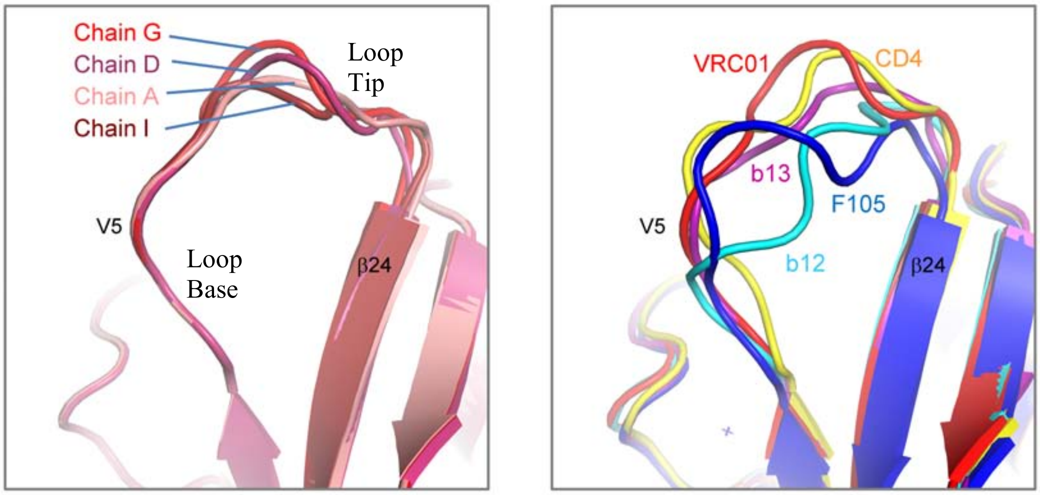

Figure S6. Conformational variation of gp120 loopV5.
Side-by-side comparison of conformation variation at the HIV-1 gp120 variable loop 5 region indicates that the four gp120 components (left panel, chains G, A, D and I) of the VRC01:gp120 complexes in the crystallographic asymmetric unit vary only at the tip of V5 loop and conformation of the V5 base is less flexible due to increased contacts by VRC01. In contrast, variation of V5 conformations in other gp120 complexes (right panel) with CD4 and CD4-binding site antibodies, F105, b12 and b13, spans over the whole range of V5 loop.

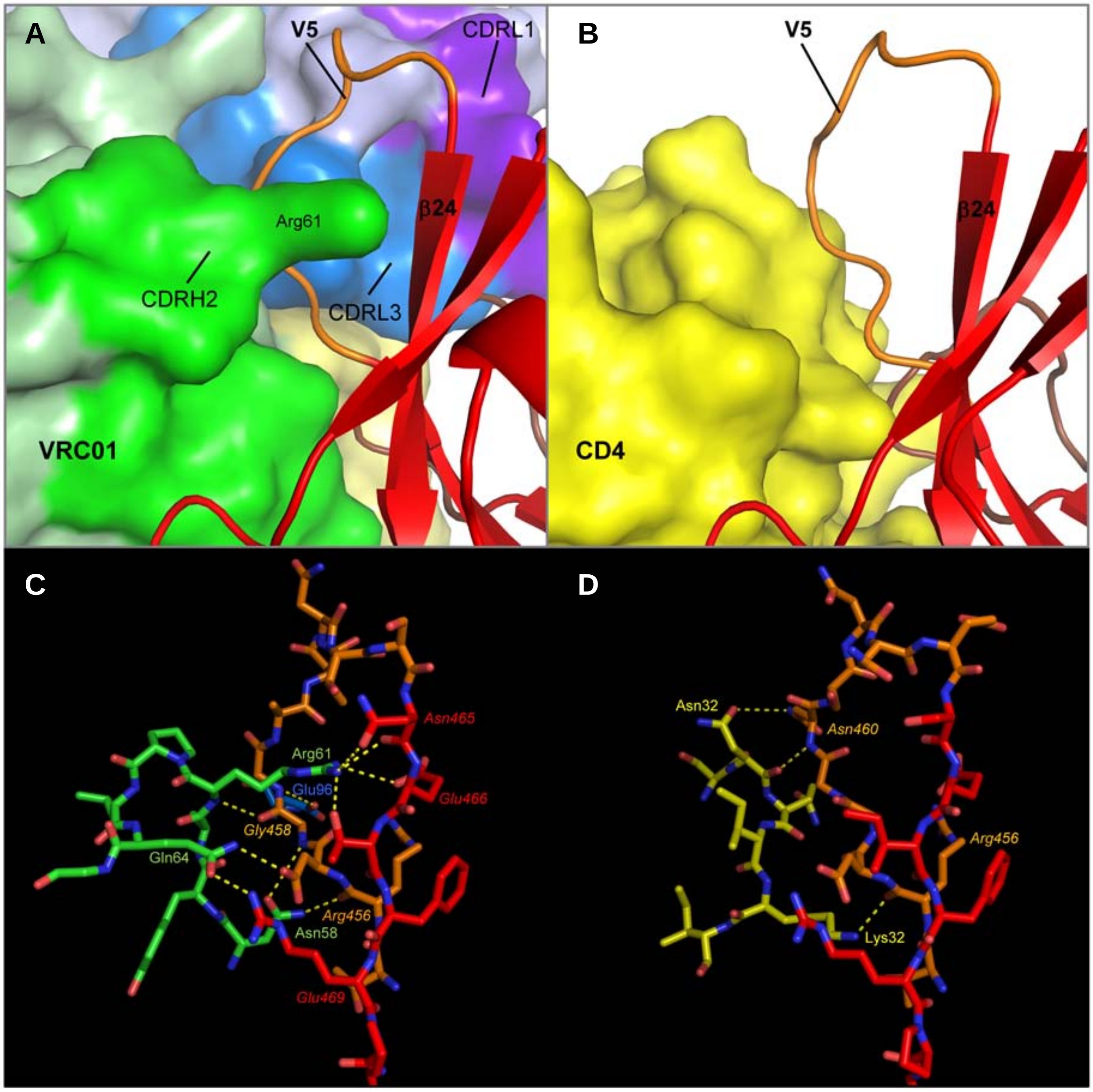

Figure S7. VRC01 recognition of V5 and β24 of gp120 is different from that of CD4

A and B: V5 loop is wedged in the gap formed by the heavy and light chains of VRC01, meanwhile, Arg61 in the CDRH2 penetrates into the cavity formed by gp120 V5 and β24, locking V5 into a less flexible conformation. In contrast, CD4 only interacts with the "front side" of V5. C and D: VRC01 engages extensive interactions with V5 and β24 with 10 hydrogen bonds and a salt bridge from both CDR H2 and CDR L3. While heavy chain Asn68, Gln64 and light chain Glu96 grab the front side of V5, heavy chain Arg61 goes behind the V5 and provides 4 hydrogen bonds to residues on β24. CD4, however, only has 3 hydrogen bonds to 2 V5 residues. It is worth to note that VRC01 only interacts with residues at the base of V5 loop and avoids the loop tip which has higher degree of sequence variation. Carbon atoms of V5 loop and β24 are shown in orange and red. The VRC01 CDRs are shown in green (H2), paleyellow (H3), purpleblue (L1) and marine (L3). Hydrogen bonds are colored yellow. Selected gp120 residues are labeled in italic.

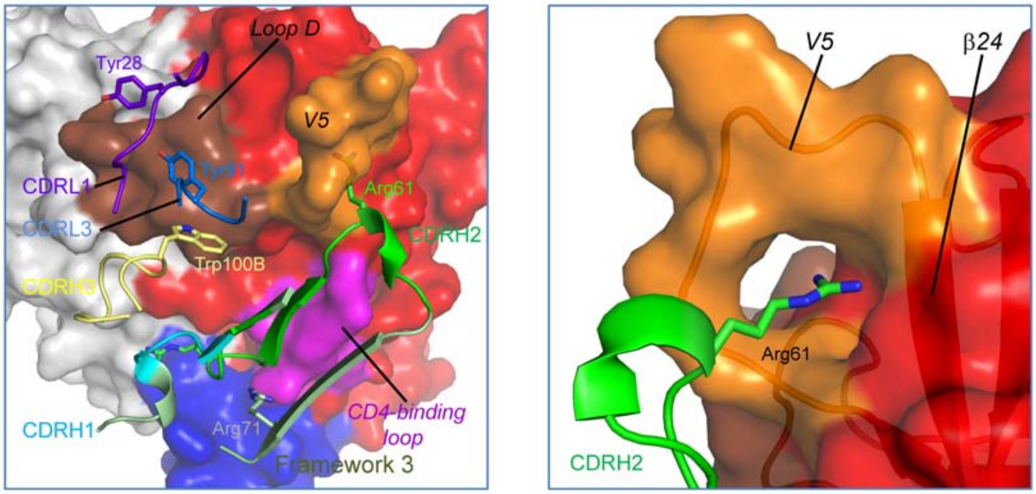

Figure S8. Key interface regions of the gp120:VRC01 complex.

gp120-interacting complementarity-determining regions (CDRs) of VRC01 are projected over the gp120 surface (left panel). Both heavy and light chains are involved in binding of gp120, mainly to the conformationally invariant outer domain (red with the CD4-binding loop, Loop D and V5 colored in magenta, brown and orange). The CDR H2 (green) spans over the CD4-binding loop and the V5/β24, with Arg61 penetrating the V5/β24 cavity (right panel). Arg71 in the framework 3 (palegreen) forms salt bridges with a conserved Asp368 in the CD4-binding loop of gp120. The light chain CDRL1 (purpleblue) and CDRL3 (marine) provide interactions to V5, loop D as well as the Loop D attached N-acetyl-glucosamine of a N-linked glycan.

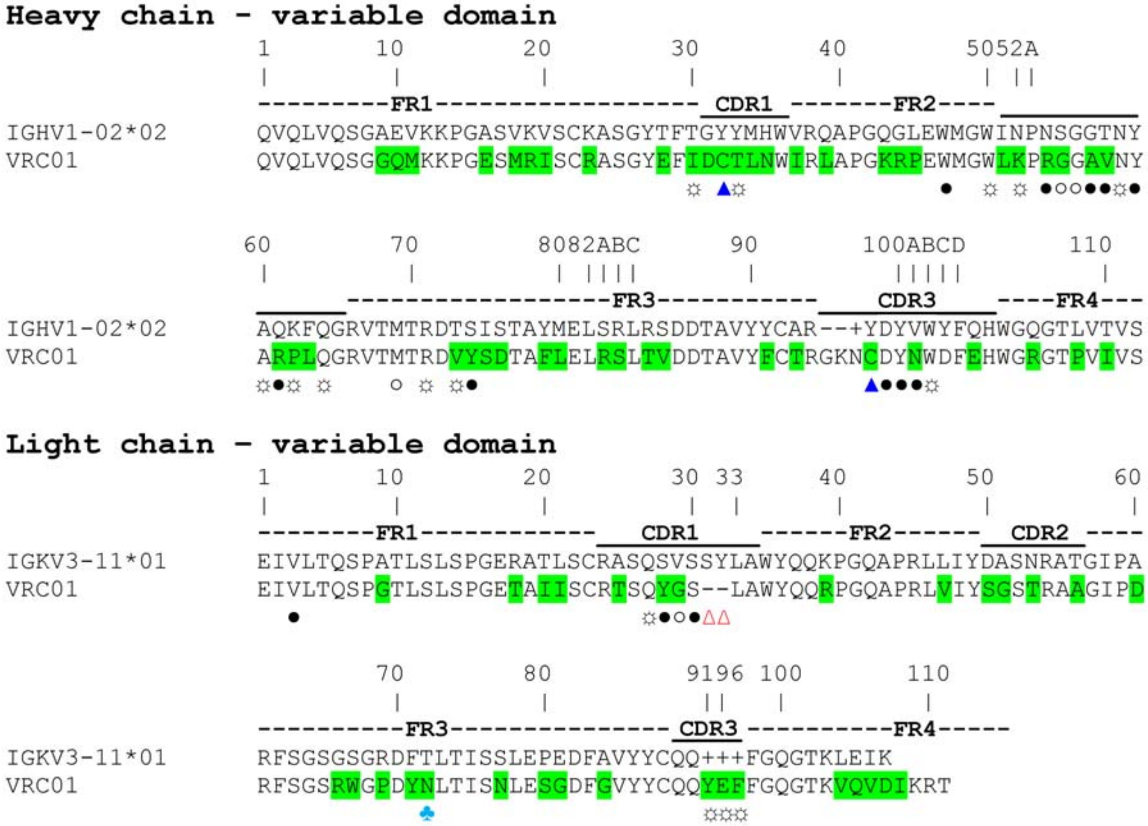

Figure S9. VRC01 sequence, gp120 contacting-sites and extent of affinity maturation. The sequence of VRC01 is shown along with nearest V_H- and $V_{K/\lambda}$-genomic precursors for heavy and light chain, respectively. Affinity maturation changes are indicated in green, with residues involved in interaction with HIV-1 gp120 highlighted by "●", if involved in both main- and side-chain interactions, by "○" if main chain-only, and by "☼" if side chain-only. "♣" marks a site of N-linked glycosylation, "▲" for Cysteine residues involved in a non-canonical disulfide, and "Δ" if the residue has been deleted during affinity maturation.

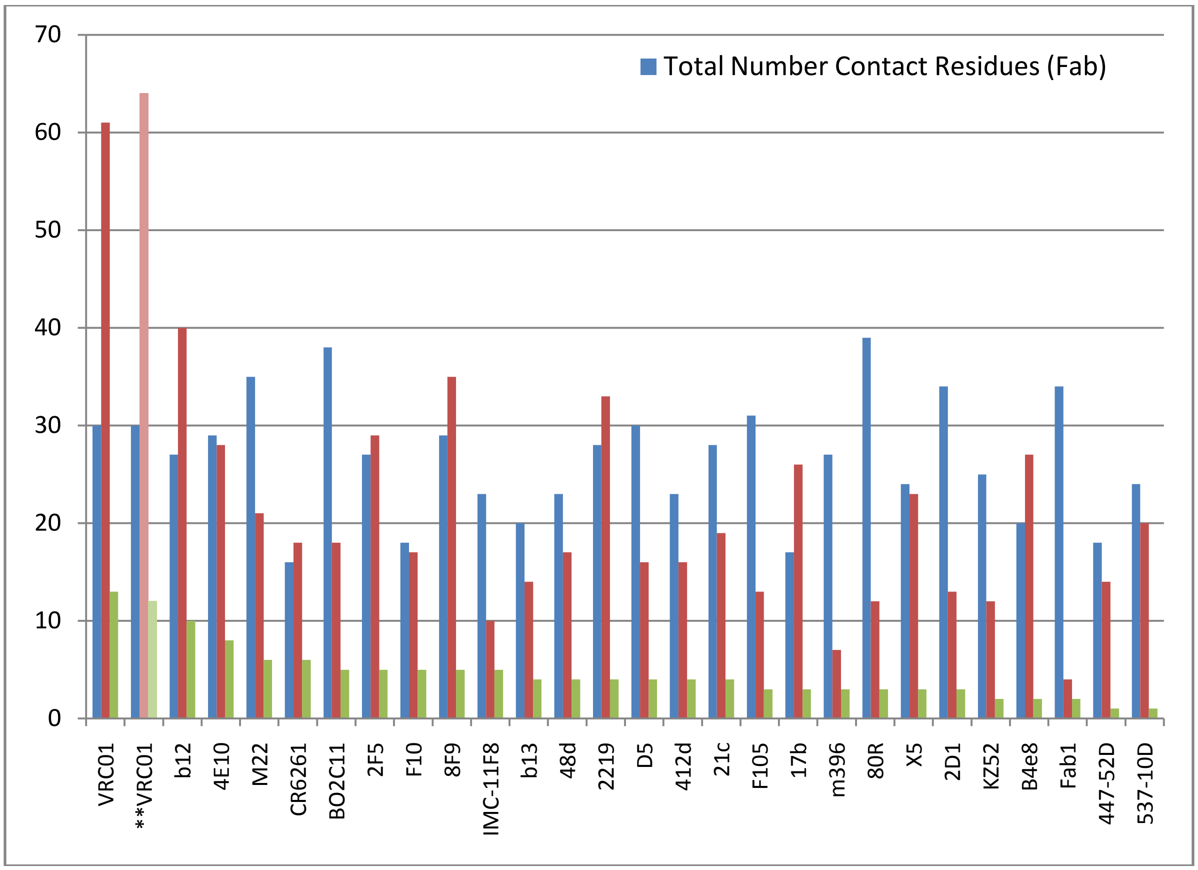

Figure S10. Contact, V-gene mutated, and mutated contact residues for the set of 26 antibodies and VRC01.

Any antibody residue in contact with the antigen in the complex is included towards the total number of contact residues (blue). The number of mutations from germline for the Vh and Vl/Vk, as well as the number of mutated contact residues are shown in red and green, respectively. The number of mutations excludes insertions and deletions. Germline alignment was performed using the amino acid sequences for the antibody heavy and light chains. For comparison, also shown is the number of mutated residues (total and only contact residues) when germline alignment is performed for the **VRC01 nucleotide sequence.

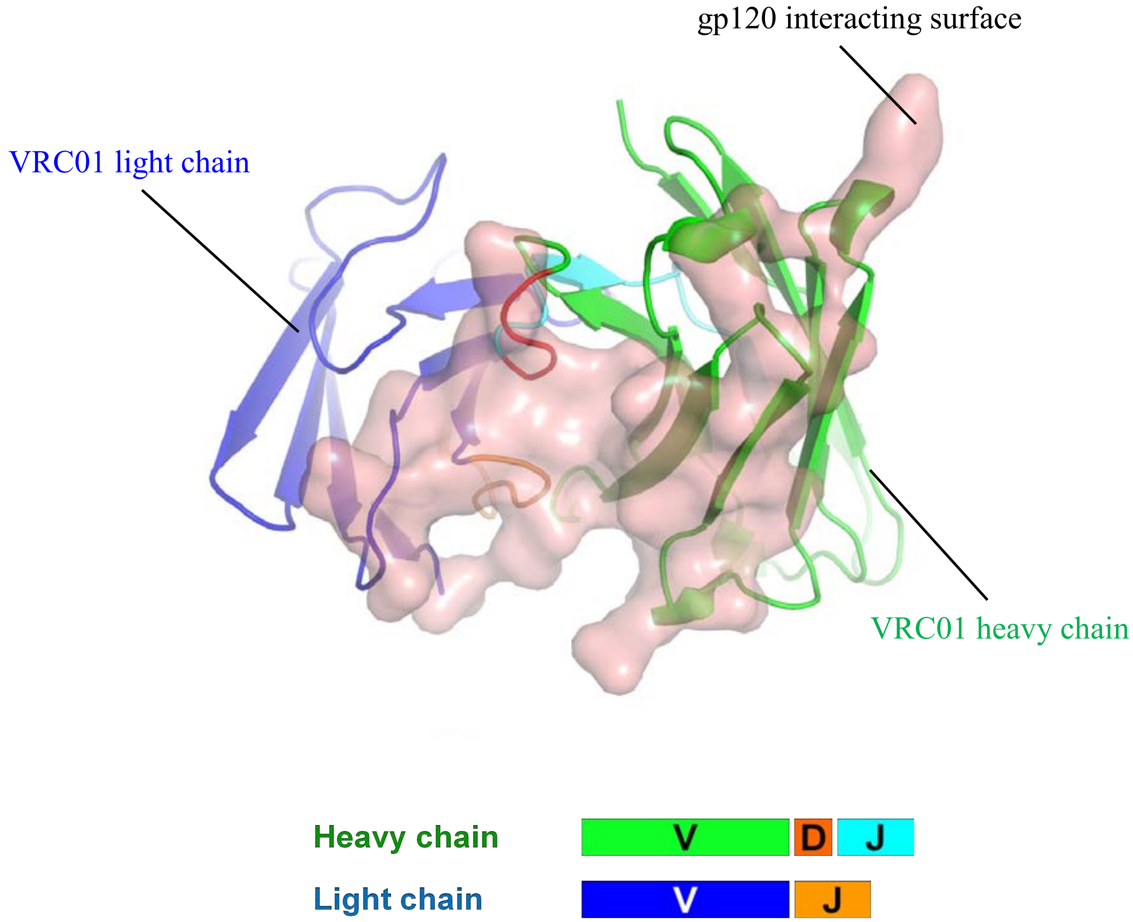

Figure S11. Contributions of VRC01 V_H-D-J and V_K-J fragments to the binding of HIV-1 gp120.

The variable domains of VRC01 are shown in cartoon diagram and the gp120 binding areas are masked with pink surface. The V_H, D_H and J_H segments are colored in green, red and cyan. The light chain V_K and J_K segments are colored in blue and orange, respectively.

```
VRC01         QVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNY
IGHV1-2*02    ........AEV....A.VKV..K....T.TGYYMH.V.Q...QGL.....IN.NS.GT..
IGHV1-2*04    ........AEV....A.VKV..K....T.TGYYMH.V.Q...QGL.....IN.NS.GT..
IGHV1-2*03    ........AEV..L.A.VKV..K....T.TGYYMH.VXQ...QGL.....IN.NS.GT..
IGHV1-3*01    ........AEV....A.VKV..K....T.TSYAMH.V.Q...Q.L.....INAGN.NTK.
IGHV1-2*01    ........AEV....A.VKV..K....T.TGYYMH.V.Q...QGL....RIN.NS.GT..
IGHV1-8*01    ........AEV....A.VKV..K....T.TSYDI..V.Q.T.QGL.....MN.NS.NTG.
IGHV1-3*02    ........AEV....A.VKV..K....T.TSYAMH.V.Q...Q.L.....SNAGN.NTK.
IGHV1-46*03   ........AEV....A.VKV..K....T.TSYYMH.V.Q...QGL....IIN.S..STS.
IGHV1-46*02   ........AEV....A.VKV..K....T.NSYYMH.V.Q...QGL....IIN.S..STS.
IGHV1-46*01   ........AEV....A.VKV..K....T.TSYYMH.V.Q...QGL....IIN.S..STS.

VRC01         ARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTR
IGHV1-2*02    .QKF.........TSIS..YM..SR.RS......Y.A.  200/293
IGHV1-2*04    .QKF..W......TSIS..YM..SR.RS......Y.A.  199/293
IGHV1-2*03    .QKF.........TSIS..YM..SR.RS......Y.A.  198/293
IGHV1-3*01    SQKF.....I...TSAS..YM..S..RSE.....Y.A.  196/293
IGHV1-2*01    .QKF.....S...TSIS..YM..SR.RS...V..Y.A.  196/293
IGHV1-8*01    .QKF........NTSIS..YM..S..RSE.....Y.A.  197/296
IGHV1-3*02    SQEF.....I...TSAS..YM..S..RSE.M...Y.A.  195/293
IGHV1-46*03   .QKF.........TSTS.VYM..S..RSE.....Y.A.  195/293
IGHV1-46*02   .QKF.........TSTS.VYM..S..RSE.....Y.A.  194/293
IGHV1-46*01   .QKF.........TSTS.VYM..S..RSE.....Y.A.  194/293
```

Figure S12a. Alignment of VRC01 Vh to the ten closest germline genes.
Results were obtained from IgBLAST using the VRC01 Vh nucleotide sequence. Residue identities are shown as dots. VRC01 residues contacting gp120 are colored: residues conserved in all ten germline genes are shown in red; residues conserved in the top match (IGHV1-2*02) but mutated in at least one of the other germline genes are shown in green; residues mutated in IGHV1-2*02 are shown in blue. The nucleotide identity fraction for each of the ten germline genes, as reported by IgBLAST, is also shown.

```
VRC01         EIVLTQSPGTLSLSPGETAIISCRTSQYG---SLAWYQQRPGQAPRLVIYSGSTRAAGIP
IGKV3-NL1*01  ........A........R.TL...A..SVSS-Y......K.......L..GA....T...
IGKV3-11*01   ........A........R.TL...A..SVSS-Y......K.......L..DA.N..T...
IGKV3-11*02   ........A........R.TL...A..SVSS-Y......K.......L..DA.N..T...
IGKV3D-11*01  ........A........R.TL...A..GVSS-Y......K.......L..DA.N..T...
IGKV3-20*01   .................R.TL...A..SVSSSY......K.......L..GA.S..T...
IGKV3-NL2*01  ........A........R.TL...A..GVSS-Y......K.......L..DA.S..T...
IGKV3-NL5*01  ........A........R.TL...A..SVSSSY......K.......L..DA.S..T...
IGKV3D-20*01  ........A........R.TL..GA..SVSSSY......K..L....L..DA.S..T...
IGKV3-NL4*01  ........A........R.TL...A..GVSS-N......K.......L..DA.N..T...
IGKV3D-15*01  ...M....A...V....R.TL...A..SVSS-N......K.......L..GA....T...

VRC01         DRFSGSRWGPDYNLTISNLESGDFGVYYCQQ
IGKV3-NL1*01  A.....GS.TEFT....S.Q.E..A.....X  218/268
IGKV3-11*01   A.....GS.T.FT....S..PE..A......  219/270
IGKV3-11*02   A.....GS.R.FT....S..PE..A......  218/270
IGKV3D-11*01  A.....GP.T.FT....S..PE..A......  217/270
IGKV3-20*01   ......GS.T.FT....R..PE..A......  219/274
IGKV3-NL2*01  A.....GP.T.FT....S..PE..A.....X  215/268
IGKV3-NL5*01  ......GS.T.FT....R..PE..A......  217/273
IGKV3D-20*01  ......GS.T.FT....R..PE..A......  217/274
IGKV3-NL4*01  A.....GP.T.FT....S..PE..A......  215/270
IGKV3D-15*01  A.....GS.TEFT....S.Q.E..A......  215/271
```

Figure S12b. Alignment of VRC01 Vk to the ten closest germline genes.

Results were obtained from IgBLAST using the VRC01 Vk nucleotide sequence up to amino acid residue Q90; since IGKV3-NL1*01 is ‘not localized’, IGKV3-11*01 was selected as a top match for the Vk germline. When comparing to IGKV3-11*01 using nucleotide sequences, the two-residue deletion in VRC01 Vk aligns to two S residues and involves a neighboring S->Y mutation. Residue identities are shown as dots. VRC01 residues contacting gp120 are colored: residues conserved in all ten germline genes are shown in red; residues conserved in IGKV3-11*01 but mutated in at least one of the other germline genes are shown in green; residues mutated in IGKV3-11*01 are shown in blue. The nucleotide identity fraction for each of the ten germline genes, as reported by IgBLAST, is also shown.

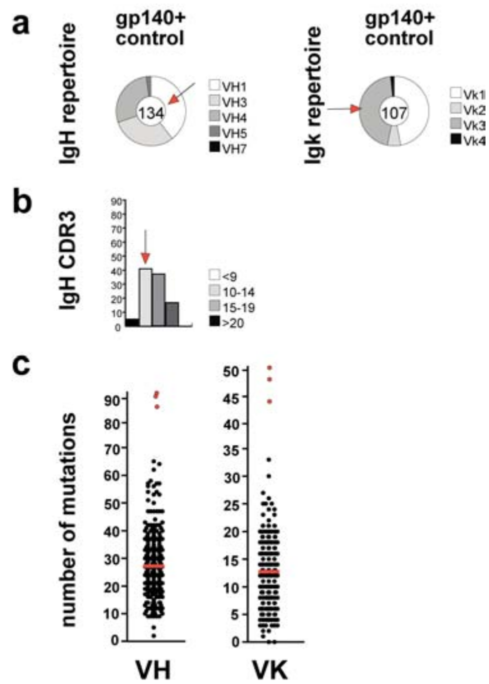

Figure S13. Sequence comparison of VRC01, VRC02 and VRC03 to a collection of gp140-binding antibodies.
(a) V_H and V_k repertoire analysis for a collection of gp140-binding antibodies and VRC01, 02 and 03. The pie charts display the distribution of V gene usage among the collection of unique antibodies. VRC01, 02, 03 genes are indicated with red arrows. b) CDR amino acid length of the collection of antibodies compared to VRC01, 02 and 03 indicated with a red arrow. CDR3 lengths were determined according to NCBI IgBlast nomenclature with the CDR3 region starting after the CTR and CVR amino acids for VRC01, 02 and 03, respectively. c) Number of nucleotide mutations in the V genes of the antibody collection compared to VRC01, 02 and 03 (indicated with red circles).

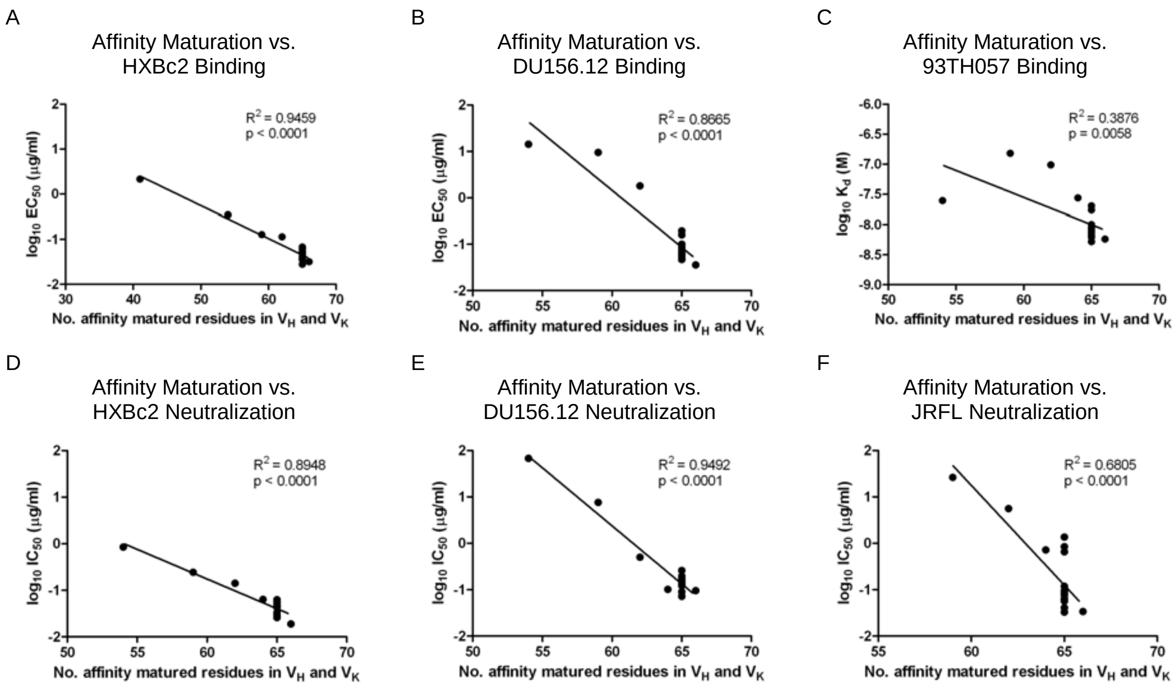

Figure S14. Correlations between the number of affinity matured residues in V_H and V_k and SPR determined dissociation constants, ELISA (EC_{50}) binding, and neutralization (IC_{50}) data for a set of VRC01 variants.

VRC01 variants were made to revert interface residues to their corresponding V_H- and V_k-germline-encoded residues in a series of single- , 4-, 7- or 12-residue mutations.

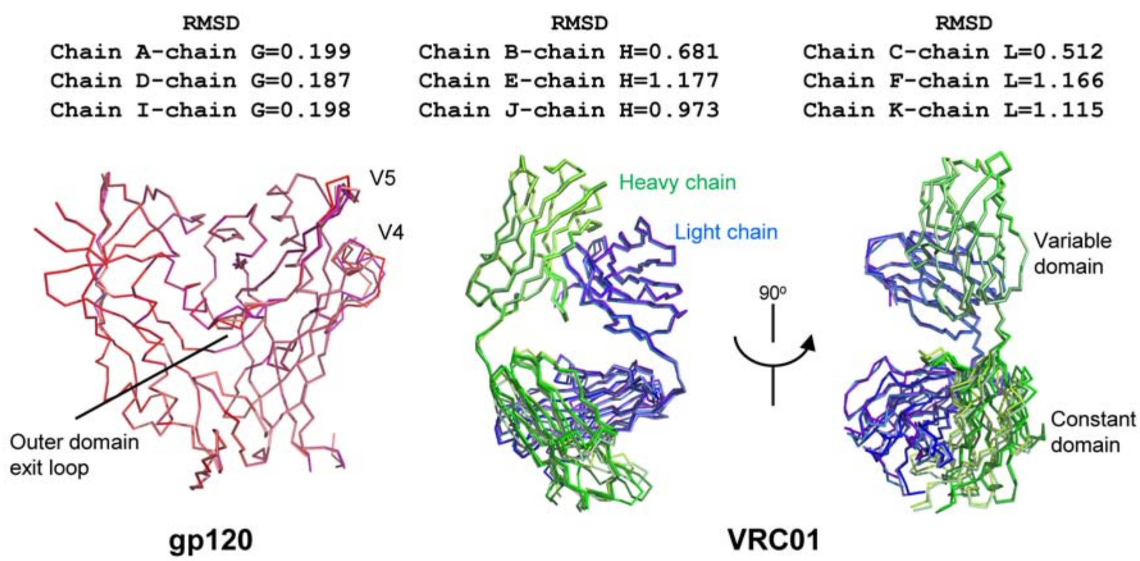

Figure S15. Comparison of the four gp120:VRC01 complexes in the asymmetric unit. The four copies of VRC01-gp120 complex in the asymmetric unit closely resemble each other. The Cα-RMSDs for all gp120 components (colored in shades of red) are below 0.2 Å. Difference in loop D, variable loop 4, variable loop 5 and the outer domain exit-loop of gp120 are noticeable. Even though the overall Cα-RMSDs for antibody VRC01 (greens for heavy chains and blues for light chains) in the complexes are around 1 Å, those for the variable domains are below 0.2 Å, variation of elbow angles in different copies of antibody VRC01 cause the constant domains assume slightly different orientation. In one of the complexes, the constant domain of VRC01 (chains E and F) is not well defined due to poor electron map density, removing it from the refinement, however, caused the refinement statistics to go worse.

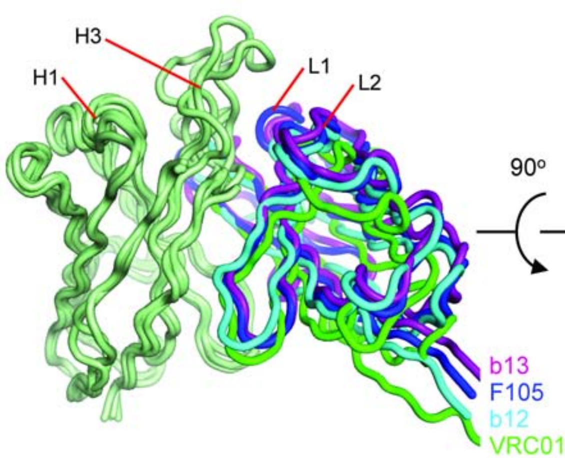

Figure S16. Comparison of the packing of heavy and light chains of VRC01 with other antibodies

Four CD4-binding site antibodies, b12, b13, F105 and VRC01 were superimposed with their heavy chain variable domains. No unusual orientations of their light chains were observed. The elbow angles, however, varies from 130 to 220 degrees due to crystal lattice packing. The superimposed heavy chains were colored palegreen and the light chains were colored green, cyan, blue and purple for VRC01, b12, F105 and b13, respectively. The CDR loops were labeled to orient the views.

Table S1. X-ray crystallographic data and refinement statistics for the antigen-binding fragment of VRC01 in complex with HIV-1 93TH057 gp120.

Crystal	93TH057-VRC01
Data collection	
Space group	P21
Wavelength, Å	0.82656
Unit cell dimensions	
a (Å)	108.6
b (Å)	98.3
c (Å)	205.3
α, β, γ (^{o})	90.0, 99.7, 90.0
Complexes per ASU	4
Resolution, Å	2.9
Completeness, %*	94.8 (68.1)
Redundancy	3.4 (1.9)
No. of total reflections	307164
No. of unique reflections	90528
I/σ*	18.6 (1.2)
R_{sym}*,†	8.0 (61.2)
Refinement statistics ($\|F\|>0\ \sigma$)	
Resolution, Å	2.9
R_{work}/R_{free}, %‡,§	19.7/25.6
RMSD bond length, Å	0.002
RMSD bond angles, °	0.534
Average B-factor, $Å^2$	57.3
Ramachandran analysis	
Favored, %	91.2
Allowed, %	99.1
PDB ID	3NGB

* Values in parentheses are for the highest resolution shell.

† $R_{sym}=\Sigma|I-\langle I\rangle|/\Sigma\langle I\rangle$, where I is the observed intensity, and <I> is the average intensity of multiple observations of symmetry related reflections.

-‡ $R=\Sigma_{hkl}||F_{obs}|-|F_{calc}||/\Sigma_{hkl}|F_{obs}|$

§ R_{free} calculated from 5% of the reflections excluded from refinement.

Table S2. Contact areas at the interface of gp120 and VRC01.

	Interface on VRC01 (Å ^{2})							Interface on gp120 (Å ^{2})
	N-term	CDR1	FR2	CDR2	FR3	CDR3	Subtotal	
Heavy chain	0	21	23	621	106	123	894	882
Kappa chain	38	123	0	0	0	190	351	367
Total							1245	1249

Table S3. gp120-contacting areas on heavy chains of CD4-binding site antibodies and CD4.

Ligand	N-term ($Å^2$)	CDR1 ($Å^2$)	FR2 ($Å^2$)	CDR2 ($Å^2$)	FR3 ($Å^2$)	CDR3 ($Å^2$)	Total Area ($Å^2$)
F105	19	186	0	92	52	500	850
b13	0	192	0	202	0	295	689
b12	0	269	0	314	23	348	954
CD4	0	82	221	528	240	11	1082
VRC01	0	21	23	621	106	123	894

Color Key	<10%
	10-20%
	20-30%
	30-40%
	40-50%
	>50%

Table S4. Hydrogen bonds and salts bridges between gp120 and VRC01.

VRC01 atom	Dist. [Å]	gp120 atom
Hydrogen bonds*		
H:TRP 50[NE1]	2.93	G:ASN 280[OD1]
H:LYS 52[NZ]	2.87	G:ALA 281[O]
H:ASN 58[ND2]	2.77	G:ARG 456[O]
H:TYR 59[OH]	3.66	G:SER 365[O]
H:ARG 61[N]	3.18	G:GLY 458[O]
H:ARG 61[NH1]	3.31	G:ASN 465[OD1]
H:ARG 61[NH1]	3.01	G:ASN 465[O]
H:ARG 61[NH1]	2.86	G:THR 467[OG1]
H:ARG 61[NH2]	3.54	G:GLU 466[OE2]
H:GLN 64[NE2]	2.99	G:ASP 457[OD1]
H:ARG 71[NH1]	3.10	G:ASP 368[OD2]
H:ARG 71[NH2]	2.83	G:ASP 368[OD1]
H:TRP 100B[NE1]	2.55	G:ASN 279[OD1]
H:GLY 54[O]	2.72	G:ASP 368[N]
H:ASN 58[OD1]	3.62	G:GLY 458[N]
H:GLN 64[OE1]	2.74	G:ARG 469[NH2]
H:ASP 99[O]	2.74	G:LYS 282[NZ]
L:SER 30[OG]	2.93	G:NAG 776[O6]
L:SER 30[N]	3.36	G:NAG 776[O6]
L:TYR 91[OH]	3.19	G:THR 278[OG1]
L:GLU 96[OE2]	3.44	G:GLY 459[N]
Salt Bridges*		
VRC01 atom	**Dist. [Å]**	**gp120 atom**
H:ARG 61[NH2]	3.54	G:GLU 466[OE2]
H:ARG 71[NH1]	3.59	G:ASP 368[OD1]
H:ARG 71[NH1]	3.10	G:ASP 368[OD2]
H:ARG 71[NH2]	2.83	G:ASP 368[OD1]
H:ARG 71[NH2]	3.80	G:ASP 368[OD2]
H:ASP 99[OD1]	3.80	G:LYS 97[NZ]

* Detailed gp120:VRC01 interface data was calculated on the EBI PISA server (http://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver) (S*20*)

Table S5. Structural comparison of VRC01-bound gp120 and gp120s in other crystalline lattices and bound by other ligands.

Clade	Ligand(s)	PDB	Chain	$RMSD_{Com}$ (Å)[a]	$RMSD_{ID}$ (Å)	$RMSD_{OD}$ (Å)	$RMSD_{BS}$ (Å)
B (HXBc2)	CD4, 48d	3JWO	A	1.035	0.497	1.046	1.183
B (HXBc2)	CD4, 48d	3JWD	A/B	1.039/1.033	0.494/0.572	1.068/1.089	1.191/1.287
B (HXBc2)	CD4, 17b	2NXY	A	1.071	0.986	1.693	1.535
B (HXBc2)	CD4, 17b	2NXZ	A	1.100	1.009	1.688	1.581
B (YU2)	CD4, 412d	2QAD	A/E	1.103/1.104	1.203/1.210	1.393/1.156	1.264/1.297
B (HXBc2)	CD4, 17b	1G9M	G	1.145	1.263	1.767	1.645
B (HXBc2)	CD4, 17b	1G9N	G	1.147	1.091	1.875	1.811
B (JR-FL)	CD4, X5	2B4C	G	1.153	1.051	2.178	1.434
B (YU2)	F23, 17b	1YYM	G/P	1.155/1.176	1.072/1.108	1.367/1.130	1.536/1.554
B (HXBc2)	CD4, 17b	1RZJ	G	1.160	1.254	1.799	1.620
B (YU2)	CD4, 17b	1RZK	G	1.160	1.105	1.893	1.793
B (YU2)	CD4M33, 17b	1YYL	G/P	1.179/1.199	1.145/1.133	1.378/1.406	1.577/1.643
B (YU2)	[Phe23]M47, 17b	2I60	G/P	1.180/1.244	1.108/1.181	1.409/1.205	1.605/1.732
B (YU2)	CD4M47, 17b	2I5Y	G/P	1.245/1.279	1.174/1.179	1.485/1.263	1.647/1.762
B (HXBc2)	CD4, 17b	1GC1	G	1.626	1.557	2.015	2.415
C (CAP210)	CD4, 21c	3LQA[b]	G	2.197	1.282	2.995	1.831
B (HXBc2)	b12	2NY7	G	4.113	4.667	3.617	9.877
B (YU2)	F105	3HI1	G/J	4.515/4.557	2.599/2.572	5.689/5.763	20.051/19.990
B (HXBc2)	b13	3IDX	G	6.524	6.239	5.004	33.779
SIV	—	2BF1[b]	A	8.583	10.202	7.899	18.535

[a] Cα-RMSDs of a set of common residues shared by all gp120s ($RMSD_{Com}$), of inner domain ($RMSD_{ID}$), of outer domain ($RMSD_{OD}$) and of bridging sheet ($RMSD_{BS}$) were calculated after each of the gp120 structures was superimposed with the VRC01-bound gp120 structure. The common set consists of 253 residues, including residues 90-120, 204-205, 215-298, 329-355, 357-392, 395, 413-469 and 473-489. The inner domain (ID) contains three segments, residues 90-118, 206-255 and 475-492. The outer domain (OD) contains three segments, residues 256-299, 330-394, 412-421 and 437-474. The bridging sheet contains two regions, residues 119-205 and 422-436.

[b] For 3LQA, which has adopted a different residue numbering, and 2BF1, which is the gp120 from SIV, sequence alignment with 1G9M is used to derive the standard HXBc2 numbering.

Table S6. Recognition of HIV-1 gp120 by the CD4 receptor and CD4-binding-site reactive antibodies.

6a. Envelope overlapping between VH domains of antibodies and CD4 domain 1

Domain	Volume (Å^3)	Combined Volume of VH and D1 (Å^3)	Overlapping of CD4 domain 1
VRC01	15544.5	18958.07	73%
b12	16350.42	23490.12	43%
b13	16267.1	24879.65	31%
F105	15072.3	21700.42	47%
CD4 domain 1	12483.89	-	100%

6b. Rotation angles between VH domains of antibodies and CD4 domain 1

	VRC01	b12	b13	F105	CD4
VRC01		144.6	135.0	174.9	42.8
b12			16.7	53.4	110.0
b13				56.3	100.4
F105					139.7
CD4					

The degree of envelope overlapping between two domains shows how much the two superimpose onto each other. To calculate the degree of envelope overlapping between CD4-binding site antibodies and CD4 as well as rotation angles between different gp120-bound ligands, gp120 complex structures were first aligned against gp120 outer domain in the gp120:VRC01 complex. The degree of envelope overlapping with CD4 was defined as $(Vol_{VH}+Vol_{D1}-Vol_{combi})/Vol_{D1}$, where Vol_{VH} is the volume under the molecular surface of heavy chain variable domain of antibody, Vol_{D1} is the volume under the molecular surface of domain 1 of CD4, Vol_{combi} is volume under the molecular surface of combined coordinates of antibody heavy chain variable domain and CD4 domain 1 after gp120 outer domain alignment. The pair wise rotation angles between different gp120-bound antibodies were calculated by superposing their framework regions of both heavy and light chain variable domains in the outer-domain-aligned complexes. For comparison with CD4, only variable domain of heavy chain was used and the alignment was carried out as previously described (S*27*). It is obvious that VRC01 and CD4 have similar orientation of approaching to gp120 and have greater degree of envelope overlapping. All superposition were performed with CCP4 package (S*7*) and the molecular volume data were calculated with GRASP (S*24*) .

Table S7. Comparison of VRC01- and CD4-contacting areas on HIV-1 gp120.

Ligand	Inner domain and Bridging sheet	LoopD+NAG	β-15/ α-3	V5	β-24	Outer domain exit-loop	Total
VRC01 (Å^2)	160	453	208	327	46	55	1249
CD4 (Å^2)	356	228	253	154	14	81	1086

Breakdown of VRC01 epitope and CD4-binding site on gp120 indicates that VRC01 has less contact at the conformationally variable inner domain and bridging sheet, while it's interactions at the Loop D, V5 and β24 regions increase about 2-folds. This shift of binding pattern explains how VRC01 overcomes conformational masking to achieve broad and potent neutralization.

Table S8. List of pdbs corresponding to the 26 human antibody complexes used in the analysis of antibody affinity maturation.

PDB ID	Description	Similar PDBs
1IQD	Human Factor VIII C2 Domain complexed to human monoclonal BO2C11 Fab (S*28*)	
2DD8	SARS-CoV Spike Receptor-Binding Domain Complexed with Neutralizing Antibody m396 (S*29*)	
2GHW	SARS spike protein receptor binding domain in complex with a neutralizing antibody 80R (S*30*)	
2NXY	HIV-1 gp120 Envelope Glycoprotein(S334A) Complexed with CD4 and Antibody 17b (S*2*)	1G9M, 1G9N, 1GC1, 1RZJ, 1RZK, 2NXZ, 2NY0, 2NY1, 2NY2, 2NY3, 2NY4, 2NY5, 2NY6, 1YYL, 1YYM, 2I60
3G04	TSH receptor in complex with a thyroid-stimulating autoantibody M22 (S*31*)	
3GBM	Fab CR6261[1] in Complex with a H5N1 influenza virus hemagglutinin (S*32*)	3GBN
3IDX	HIV-gp120 core in complex with CD4-binding site antibody b13 (S*3*)	3IDY
3JWD	HIV-1 gp120 in complex with CD4 and Fab 48d (S*33*)	
3H3P	HIV epitope-scaffold 4E10 Fv complex (S*34*)	1TZG, 2FX7, 2FX8, 2FX9
3FKU	Influenza hemagglutinin (H5) in complex with a broadly neutralizing antibody F10 (S*35*)	
3CSY	Trimeric prefusion Ebola virus glycoprotein in complex with neutralizing antibody KZ52 (S*36*)	
1Q1J	Anti-HIV-1 Fab 447-52D in complex with V3 peptide (S*37*)	3GHB, 3C2A
1TJI	Broadly neutralizing anti-HIV-1 antibody 2F5 in complex with a gp41 17mer epitope (S*38*)	3D0L, 3D0V, 3DRQ, 3DRT, 1TJG, 1TJH, 1U8H, 1U8I, 1U8J, 1U8K, 1U8L, 1U8M, 1U8N, 1U8O, 1U8P, 1U8Q, 1U91, 1U92, 1U93, 1U95, 2F5B, 3IDG, 3IDI, 3IDJ, 3IDM, 3IDN, 2P8L, 2P8M, 2P8P, 2PW1, 2PW2
2B1H	Anti-HIV-1 V3 Fab 2219 in complex with UG29 peptide (S*39*)	2B1A, 2B0S
3GHE	Anti-HIV-1 Fab 537-10D in complex with V3 peptide (S*40*)	
3EYF	Anti-human cytomegalovirus antibody 8f9 plus gB peptide (S*41*)	
2CMR	HIV-1 neutralizing antibody D5 Fab bound to the gp41 inner-core mimetic 5-helix (S*42*)	
2NY7	HIV-1 gp120 Envelope Glycoprotein Complexed with the Broadly Neutralizing CD4-Binding-Site Antibody b12 (S*2*)	
2QAD	Tyrosine-sulfated 412d antibody complexed with HIV-1 YU2 gp120 and CD4 (S*43*)	
2QSC	Anti-HIV-1 V3-Fab F425-B4e8 in complex with a V3-peptide (S*44*)	
3B2U	Isolated domain III of the extracellular region of the epidermal growth factor receptor in complex with the Fab fragment of IMC-11F8 (S*45*)	3B2V
3H42	PCSK9 in complex with from LDLR competitive antibody Fab (S*46*)	
2B4C	HIV-1 JR-FL gp120 core protein containing the third variable region (V3) complexed with CD4 and the X5 antibody (S*47*)	
3HI1	HIV-1 gp120 (core with V3) in Complex with CD4-Binding-Site Antibody F105 (S*3*)	
3LQA	Clade C gp120 in complex with sCD4 and 21c Fab (S*48*)	
3LZF	Fab 2D1 in Complex with the 1918 Influenza Virus Hemagglutinin (S*49*)	

[1] From IgM^+ memory B cells

Table S9. List of discarded pdbs from initial IMGT/PDB results

Reason for Discarding	PDB ID(s)
not an antibody-protein or antibody-peptide complex	1GPQ, 1GWD, 1H6M, 1H87, 1HC0, 1HNI, 1HNV, 1QE1, 1N8Y, 1S6P, 1S6Q, 1S9E, 1S9G, 1SUQ, 1SV5, 1UN3, 1UN4, 1UN5, 1UUZ, 1W0Y, 1W2K, 1W6Z, 1Y0L, 1Y18, 2B5J, 2BAN, 2BE2, 2BLX, 2BLY, 2BPU, 2C4F, 2C8O, 2C8P, 2CDE, 2CDF, 2CDG, 2CGI, 2I5Y, 2ITN, 2ITO, 2ITQ, 2ITT, 2ITU, 2ITV, 2ITW, 2ITY, 2ITZ, 2J5E, 2J5F, 2J6M, 2JB5, 2JB6, 2JIT, 2JIU, 2JIV, 2O5X, 2O5Y, 2O5Z, 2VB1, 2W1L, 2W1M, 2W1X, 2W1Y, 2WAR, 3BT0, 3BT1, 3CXF, 3D87, 3FO0, 3FO1, 3FO2, 1CLY, 1FOR, 1GGB, 1GGC, 1H3T, 1H3U, 1H3V, 1H3W, 1H3X, 1HCV, 1JPT, 1K6Q, 1OL0, 1RHH, 1S5I, 1UCB, 2A6J, 2FJF, 3C08, 3CFJ, 3CFK, 3EYV, 3G6A, 3INU, 8FAB, 2WAH, 1CK0, 1TFH, 1RZG
non-human antibody	2HFG, 2HH0, 2OSL, 3BKY, 3D85, 3IU3, 1C5B, 1C5C, 3HI6, 2H9G, 3EO1, 1BJ1, 1CZ8, 1S3K, 1S78, 2EH7, 2EH8, 3C09, 3EOA, 3IXT, 1YY9, 1TZH, 1TZI, 2QQN, 3GRW, 1A3R, 1AHW, 1E6J, 1E6O, 1F90, 1FE8, 1FPT, 1GGI, 1HYS, 2HMI, 1J5O, 1KB5, 1MHH, 1N5Y, 1N6Q, 1N8Z, 1OAK, 1R0A, 1SY6, 1T03, 1V7M, 1V7N, 1YJD, 1YNT, 2ADF, 2AEP, 2AEQ, 2BDN, 2CK0, 2FD6, 2HKF, 2I5J, 2V17, 2VWE, 2ZCH, 2ZCK, 2ZCL, 2ZPK, 3BT2, 3CK0, 3CXD, 3DSF, 3FFD, 3IFL, 3IFN, 3IFO, 3IFP, 3KJ6, 1FNS, 1H0D, 1JRH, 1ACY, 1FGN, 1NAK, 2OR9, 2VXT, 3BSZ, 3G6J, 1LK3, 2ARJ, 3K2U, 2R0L, 2WUB, 1JPS, 1UJ3, 1I9R, 3BDY, 3BE1, 2FEE, 3G6D, 2VXS, 2FED, 1ZA3, 2FJG, 2FJH, 2WUC, 2VYR, 3BN9, 3DVG, 3FN0, 3DVN, 2VDN, 1W72, 2VDP, 2VDQ
non-IgG antibody	2R56, 1ADQ, 2J6E, 2VXQ, 1DEE, 1HEZ
modified antigenic target	2OQJ, 1MCC, 1MCD, 1MCE, 1MCF, 1MCH, 1MCI, 1MCJ, 1MCK, 1MCL, 1MCN, 1MCQ, 1MCR, 1MCS, 1N0X, 1MCB
Fc fragment	1FC2, 1OQO, 1OQX, 2IWG, 1FCC, 1DN2
insufficient information	2QQK, 2QQL, 2JIX, 2ZNW, 1NL0, 2ZNX, 3H0T, 3GJF, 3GJG, 3HAE

The entire PDB was searched with the terms, "Human antibody complex", and results were combined with a search of the IMGT/3Dstructure-DB database. This resulted in a total of 332 pdb entries. These were manually examined for human IgG antibodies that had undergone natural affinity maturation and contained antibody-protein or antibody-peptide complexes. Antibody complexes that fit these criteria are listed in Table S8. Antibodies that did not fit these criteria are listed here, grouped into several categories. While some structures may have multiple reasons for discarding, a single reason is reported here. The `Non-human antibody' category includes antibodies from other species, as well as humanized and chimeric antibodies, and antibodies subjected to artificial affinity maturation. The `insufficient information' category includes structures for which sufficient evidence for inclusion in the analysis could not be identified. The `modified antigenic target' category includes structures containing artificially-constructed or modified antigens for improved/altered binding to the antibody.

Table S10a. Characterization by surface-plasmon resonance and ELISA of the interaction between gp120 and variants of VRC01 IgG.

Mutant Number	Mutant category	SPR Kinetics for 93TH057 gp120			EC50 (μg/ml)*				Mutations or treatment
		ka (M/s)	kd (s^{-1})	KD (M)	93TH057	Stabilized HXBc2 [a]	HXBc2	Du156.12	
1	Single interface revertant	4.24E+04	2.81E-04	6.64E-09	0.0631	0.0461	0.028	0.048	Heavy chain: T33Y
2		1.79E+04	3.61E-04	2.02E-08	0.1501	0.0610	0.038	0.072	Heavy chain: G54S
3		6.62E+04	3.47E-04	5.24E-09	0.1287	0.0565	0.052	0.101	Heavy chain: A56G
4		2.70E+04	1.96E-04	7.27E-09	0.0515	0.0475	0.054	0.164	Heavy chain: V57T
5		5.21E+04	4.44E-04	8.53E-09	0.0372	0.0350	0.038	0.066	Heavy chain: P62K
6		4.98E+04	3.79E-04	7.61E-09	0.0604	0.0309	0.068	0.097	Heavy chain: V73T
7		3.40E+04	3.43E-04	1.01E-08	0.0419	0.0344	0.050	0.071	Heavy chain: Y74S
8		4.79E+04	3.12E-04	6.52E-09	0.1135	0.0669	0.041	0.063	Heavy chain: I30T
9		4.53E+04	3.50E-04	7.74E-09	0.0401	0.0480	0.038	0.056	Heavy chain: K52N
10		7.06E+04	4.40E-04	6.23E-09	0.0300	0.2135	0.062	0.063	Heavy chain: R53N
11		4.30E+04	2.92E-04	6.78E-09	0.0243	0.0674	0.037	0.086	Heavy chain: R61Q
12		4.73E+04	8.20E-04	1.73E-08	0.0420	0.0552	0.036	0.196	Light chain: Y30S
13	4-revertant	7225	7.02E-04	9.72E-08	0.2497	0.0665	0.114	1.80	Heavy chain: A56G ,V57T, P62K, V73T
14	7-revertant	3732	5.67E-04	1.52E-07	1.0780	0.0698	0.128	9.02	Heavy chain: T33Y, G55S, A56G, V57T, P62K, V73T, Y74S
15	12-revertant	6.32E+03	1.56E-04	2.47E-08	0.3760	0.2829	0.348	14.33	Heavy chain: I30T, K52N, R53N, G54S, A56G, V57T, R61Q, P62K, V73T, Y74S; Light chain: Y28S
16	C32AC98S	3.87E+04	3.45E-04	8.92E-09	0.0420	0.0586	0.047	0.048	Heavy chain: C32S,C98A
17	Insertion-AA	1.75E+04	5.81E-03	3.20E-07	1.2660	0.0420	0.230	2.02	Light chain insertion AlaAla after position 30
18	Insertion-SY	1.83E+04	5.01E-04	2.74E-08	0.0926	0.0658	ND [b]	ND	Light chain insertion SerTyr after position 30
19	gHC+LC	ND	ND	ND	2.2860	1.2980	21.38	>50	Germline VH and wild type Light chain
20	HC+gLC	ND	ND	ND	NBD [c]	0.1004	2.14	>50	Germline VL and wild type heavy chain
21	gHC+gLC		NBD		NBD		>50	>50	Both germline VH and VL gene
	Deglycosylated	5.49E+04	1.36E-04	2.48E-09	0.0480	0.0430	0.045	0.050	Enzyme deglycosylation to remove glycans
Wild Type	VRC01	3.83E+04	2.20E-04	5.76E-09	0.0355	0.0350	0.032	0.037	

[*]: ELISA experiments for 93TH057 and stabilized HXBc2 core gp120 were performed in different format from that of HXBc2 and Du156.12. The former ones were carried out in triplicates and the latter ones were performed without duplication.
[a]: HXBc2 core Ds12F123 with mutations to stabilize the gp120 in its CD4-bound conformation
[b]: ND: constants not determined
[c]. NBD: No binding detected

Table S10b. Neutralization IC50 values (μg/ml) of VRC01 variants against three HIV-1 primary isolates and the lab strain HXB2.

Mutant Number	Mutant Category	Q842.d12 (clade A)		JRFL (clade B)		Du156.12 (clade C)		HXB2 (clade B)		Mutations or treatment
		Mean[a]	SEM[a]	Mean[a]	SEM[a]	Mean[a]	SEM[a]	Mean[a]	SEM[a]	
1	Single interface revertant	0.028	0.003	0.041	0.003	0.125	0.002	0.046	0.001	Heavy chain: T33Y
2		0.026	0.001	0.077	0.003	0.129	0.009	0.049	0.004	Heavy chain: G54S
3		0.028	0.002	0.064	0.003	0.169	0.024	0.045	0.003	Heavy chain: A56G
4		0.028	0.002	0.850	0.076	0.148	0.039	0.054	0.000	Heavy chain: V57T
5		0.022	0.001	0.033	0.002	0.072	0.003	0.029	0.003	Heavy chain: P62K
6		0.040	0.000	0.095	0.004	0.196	0.026	0.056	0.002	Heavy chain: V73T
7		0.045	0.003	0.119	0.006	0.261	0.046	0.047	0.004	Heavy chain: Y74S
8		0.030	0.001	0.059	0.006	0.153	0.049	0.027	0.005	Heavy chain: I30T
9		0.027	0.002	0.086	0.002	0.090	0.005	0.031	0.002	Heavy chain: K52N
10		0.040	0.003	0.659	0.037	0.132	0.016	0.063	0.007	Heavy chain: R53N
11		0.024	0.003	0.062	0.012	0.171	0.037	0.026	0.0003	Heavy chain: R61Q
12		0.034	0.001	1.36	0.184	0.135	0.019	0.043	0.003	Light chain: Y30S
13	4-revertant	0.080	0.002	5.62	0.579	0.504	0.035	0.142	0.008	Heavy chain: A56G ,V57T, P62K, V73T
14	7-revertant	0.263	0.011	26.5	2.959	7.63	1.344	0.244	0.009	Heavy chain: T33Y, G55S, A56G, V57T, P62K, V73T, Y74S
15	12-revertant	0.360	0.006	>200	NA	67.7	3.655	0.850	0.122	Heavy chain: I30T, K52N, R53N, G54S, A56G, V57T, R61Q, P62K, V73T, Y74S; Light chain: Y28S
16	C32SC98A	0.027	0.003	0.057	0.002	0.123	0.043	0.033	0.004	Heavy chain: C32S,C98A
17	Insertion-AA	0.319	0.026	2.66	0.360	5.02	1.741	2.442	1.091	Light chain insertion AlaAla after position 30
18	Insertion-SY	0.023	0.001	0.721	0.135	0.102	0.007	0.064	0.002	Light chain insertion SerTyr after position 30
19	gHC+LC	>500	NA	>500	NA	>500	NA	>500	NA	Germline VH and wild type light chain
20	HC+gLC	>500	NA	>500	NA	>500	NA	>500	NA	Germline VL and wild type heavy chain
21	gHC+gLC	>500	NA	>500	NA	>500	NA	>500	NA	Both germline VH and VL gene
22	Deglycosylated	0.028	0.002	0.031	0.002	0.059	0.003	0.022	0.002	Enzyme deglycosylation to remove glycans
Wild Type	VRC01	0.026	0.002	0.034	0.004	0.096	0.013	0.019	0.003	

[a]: Mean and SEM were calculated from three independent experiments; NA: not applicable

Table S11. Correlations between binding and neutralization data for VRC01 variants and panels of gp120 and HIV-1 isolates.

	Kd (93TH057)	EC_{50} (93TH057)	EC_{50} (Stabilized HXBc2)	EC_{50} (HXBc2)	EC_{50} (DU156.12)	IC_{50} (HXB2)	IC_{50} (Q842.d12)	IC_{50} (JRFL)	IC_{50} (Du156.12)
Kd (93TH057)		0.7178	0.01568	0.5093	0.6444	0.6738	0.5923	0.6675	0.4563
EC_{50} (93TH057)			0.04609	0.627	0.7025	0.7213	0.7192	0.4483	0.6354
EC_{50} (Stabilized HXBc2)				0.2432	0.2106	0.1394	0.1655	0.1198	0.26
EC_{50} (HXB2)					0.8162	0.8584	0.8888	0.5898	0.851
EC_{50} (DU156.12)						0.7347	0.8738	0.8348	0.8756
IC_{50} (HXB2)							0.8572	0.5223	0.7742
IC_{50} (Q842.d12)								0.588	0.9192
IC_{50} (JRFL)									0.5796
IC_{50} (Du156.12)									

Correlations (from linear regression analysis) were obtained for all pairs of binding and neutralization data. R^2 values are shown colored by p-values: red for $p > 0.05$, yellow for $0.01 < p < 0.05$, and white for $p < 0.01$. Germline variants were not included in the correlation analysis since precise data were not available for the majority of the experiments.

Table S12. Interactions between heavy chain of VRC01 and gp120.

<table>
<thead>
<tr><th></th><th>Interface residue</th><th>Bond Type*</th><th>ASA*</th><th>BSA*</th><th></th><th>$\Delta^{i}G$</th></tr>
</thead>
<tbody>
<tr><td rowspan="24">Heavy Chain</td><td>H:ILE 30</td><td></td><td>65.14</td><td>17.92</td><td>|||</td><td>0.29</td></tr>
<tr><td>H:THR 33</td><td></td><td>27.12</td><td>2.62</td><td>|</td><td>0.03</td></tr>
<tr><td>H:TRP 47</td><td></td><td>67.02</td><td>23.16</td><td>||||</td><td>0.37</td></tr>
<tr><td>H:TRP 50</td><td>H</td><td>45.64</td><td>41.10</td><td>||||||||||</td><td>0.30</td></tr>
<tr><td>H:LYS 52</td><td>H</td><td>80.95</td><td>40.21</td><td>|||||</td><td>-1.16</td></tr>
<tr><td>H:ARG 53</td><td></td><td>151.61</td><td>62.69</td><td>|||||</td><td>0.02</td></tr>
<tr><td>H:GLY 54</td><td>H</td><td>69.07</td><td>53.07</td><td>||||||||</td><td>0.07</td></tr>
<tr><td>H:GLY 55</td><td></td><td>20.23</td><td>17.16</td><td>|||||||||</td><td>-0.00</td></tr>
<tr><td>H:ALA 56</td><td></td><td>48.64</td><td>33.08</td><td>|||||||</td><td>0.52</td></tr>
<tr><td>H:VAL 57</td><td></td><td>52.88</td><td>40.45</td><td>||||||||</td><td>0.24</td></tr>
<tr><td>H:ASN 58</td><td>H</td><td>67.15</td><td>65.13</td><td>||||||||||</td><td>-0.53</td></tr>
<tr><td>H:TYR 59</td><td>H</td><td>51.57</td><td>36.62</td><td>||||||||</td><td>0.19</td></tr>
<tr><td>H:ALA 60</td><td></td><td>12.55</td><td>10.71</td><td>|||||||||</td><td>0.17</td></tr>
<tr><td>H:ARG 61</td><td>HS</td><td>209.87</td><td>154.57</td><td>||||||||</td><td>-0.77</td></tr>
<tr><td>H:PRO 62</td><td></td><td>106.31</td><td>18.74</td><td>||</td><td>0.30</td></tr>
<tr><td>H:GLN 64</td><td>H</td><td>104.46</td><td>47.50</td><td>|||||</td><td>-0.65</td></tr>
<tr><td>H:MET 69</td><td></td><td>6.86</td><td>0.98</td><td>||</td><td>-0.01</td></tr>
<tr><td>H:ARG 71</td><td>HS</td><td>71.23</td><td>25.47</td><td>||||</td><td>-0.71</td></tr>
<tr><td>H:VAL 73</td><td></td><td>54.83</td><td>23.26</td><td>|||||</td><td>0.37</td></tr>
<tr><td>H:TYR 74</td><td></td><td>212.55</td><td>57.27</td><td>|||</td><td>0.64</td></tr>
<tr><td>H:ASP 99</td><td>HS</td><td>131.87</td><td>47.84</td><td>||||</td><td>-0.13</td></tr>
<tr><td>H:TYR 100</td><td></td><td>116.82</td><td>19.39</td><td>||</td><td>0.31</td></tr>
<tr><td>H:ASN 100A</td><td></td><td>22.19</td><td>13.02</td><td>||||||</td><td>0.14</td></tr>
<tr><td>H:TRP 100B</td><td>H</td><td>114.38</td><td>42.37</td><td>||||</td><td>0.12</td></tr>
<tr><td rowspan="32">gp120</td><td>G:LYS 97</td><td>S</td><td>146.28</td><td>29.09</td><td>||</td><td>-1.04</td></tr>
<tr><td>G:THR 123</td><td></td><td>47.04</td><td>0.16</td><td>|</td><td>0.00</td></tr>
<tr><td>G:GLY 124</td><td></td><td>76.25</td><td>39.85</td><td>||||||</td><td>0.54</td></tr>
<tr><td>G:GLY 198</td><td></td><td>90.93</td><td>12.42</td><td>||</td><td>-0.11</td></tr>
<tr><td>G:ASN 279</td><td>H</td><td>63.04</td><td>39.40</td><td>|||||||</td><td>-0.34</td></tr>
<tr><td>G:ASN 280</td><td>H</td><td>67.85</td><td>41.02</td><td>|||||||</td><td>-0.46</td></tr>
<tr><td>G:ALA 281</td><td>H</td><td>85.48</td><td>74.66</td><td>|||||||||</td><td>0.73</td></tr>
<tr><td>G:LYS 282</td><td>H</td><td>68.01</td><td>30.24</td><td>|||||</td><td>-0.71</td></tr>
<tr><td>G:SER 365</td><td>H</td><td>98.84</td><td>64.53</td><td>|||||||</td><td>0.31</td></tr>
<tr><td>G:GLY 366</td><td></td><td>43.38</td><td>23.29</td><td>||||||</td><td>0.15</td></tr>
<tr><td>G:GLY 367</td><td></td><td>58.12</td><td>21.26</td><td>||||</td><td>0.27</td></tr>
<tr><td>G:ASP 368</td><td>HS</td><td>78.72</td><td>49.32</td><td>|||||||</td><td>-0.46</td></tr>
<tr><td>G:ILE 371</td><td></td><td>56.48</td><td>49.29</td><td>|||||||||</td><td>0.79</td></tr>
<tr><td>G:TRP 427</td><td></td><td>56.37</td><td>10.66</td><td>||</td><td>-0.12</td></tr>
<tr><td>G:GLN 428</td><td></td><td>38.47</td><td>0.37</td><td>|</td><td>-0.00</td></tr>
<tr><td>G:GLY 429</td><td></td><td>45.93</td><td>6.93</td><td>||</td><td>0.11</td></tr>
<tr><td>G:THR 430</td><td></td><td>118.00</td><td>57.89</td><td>|||||</td><td>0.</td></tr>
<tr><td>G:THR 455</td><td></td><td>50.80</td><td>35.29</td><td>|||||||</td><td>0.51</td></tr>
<tr><td>G:ARG 456</td><td>H</td><td>29.79</td><td>3.93</td><td>||</td><td>-0.04</td></tr>
<tr><td>G:ASP 457</td><td>H</td><td>50.53</td><td>48.22</td><td>||||||||||</td><td>0.14</td></tr>
<tr><td>G:GLY 458</td><td>H</td><td>50.68</td><td>44.48</td><td>|||||||||</td><td>-0.22</td></tr>
<tr><td>G:GLY 459</td><td></td><td>87.35</td><td>37.81</td><td>|||||</td><td>0.40</td></tr>
<tr><td>G:ALA 460</td><td></td><td>75.49</td><td>28.45</td><td>||||</td><td>0.22</td></tr>
<tr><td>G:THR 463</td><td></td><td>53.37</td><td>16.26</td><td>||||</td><td>0.20</td></tr>
<tr><td>G:ASN 465</td><td>H</td><td>39.19</td><td>6.74</td><td>||</td><td>-0.10</td></tr>
<tr><td>G:GLU 466</td><td>HS</td><td>21.07</td><td>6.58</td><td>||||</td><td>-0.07</td></tr>
<tr><td>G:THR 467</td><td>H</td><td>24.23</td><td>11.16</td><td>|||||</td><td>-0.13</td></tr>
<tr><td>G:ARG 469</td><td>H</td><td>46.99</td><td>21.29</td><td>|||||</td><td>-0.53</td></tr>
<tr><td>G:GLY 472</td><td></td><td>16.47</td><td>7.83</td><td>|||||</td><td>-0.09</td></tr>
<tr><td>G:GLY 473</td><td></td><td>42.85</td><td>27.54</td><td>|||||||</td><td>-0.06</td></tr>
<tr><td>G:ASP 474</td><td></td><td>66.09</td><td>19.18</td><td>|||</td><td>-0.02</td></tr>
<tr><td>G:LYS 476</td><td></td><td>60.24</td><td>3.72</td><td>|</td><td>-0.03</td></tr>
</tbody>
</table>

*** Bond type:** H: **H**ydrogen, D **D**isulphide bond, S: **S**alt bridge C: **C**ovalent link
ASA Accessible Surface Area, Å²
BSA Buried Surface Area, Å²
Δ^{i}**G** Solvation energy effect, kcal/mol
|||| Buried area percentage, one bar per 10%
Detailed gp120:VRC01 interface data was calculated on the EBI PISA server (http://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver) (S*20*)

Table S13. Interactions between light chain of VRC01 and gp120.

	Interface residue	Bond type*	ASA*	BSA*		$\Delta^{i}G$
Light chain	L:VAL 3		117.89	37.56	||||	0.07
	L:GLN 27		124.49	24.07	||	-0.15
	L:TYR 28		186.13	77.44	|||||	0.39
	L:GLY 29		20.78	4.89	|||	0.08
	L:SER 30	H	71.56	19.97	|||	-0.03
	L:TYR 91	H	130.33	80.71	|||||||	0.60
	L:GLU 96	H	126.90	58.83	|||||	-0.30
	L:PHE 97		102.41	50.10	|||||	0.80
gp120	G:ASN 276		85.13	24.64	|||	-0.26
	G:THR 278	H	121.24	83.13	|||||||	0.60
	G:ASN 279		63.04	23.64	||||	0.17
	G:ASN 280		67.85	26.83	||||	-0.31
	G:ARG 456		29.79	1.91	|	-0.06
	G:GLY 458		50.68	5.28	||	0.08
	G:GLY 459	H	87.35	31.46	||||	-0.04
	G:ALA 460		75.49	8.01	||	-0.03
	G:ASN 461		152.02	65.92	|||||	0.06
	G:NAG 776	H	349.97	96.51	|||	-1.92

*** Bond type:** H: **H**ydrogen, D **D**isulphide bond, S: **S**alt bridge C: **C**ovalent link
ASA Accessible Surface Area, Å²
BSA Buried Surface Area, Å²
Δ^{i}G Solvation energy effect, kcal/mol
|||| Buried area percentage, one bar per 10%

Detailed gp120:VRC01 interface data was calculated on the EBI PISA server (http://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver) (S*20*)

Table S14. Neutralization by VRC01 and CD4-Ig against a panel of 190 Env pseudoviruses representing all major circulating clades of HIV-1.

		Measured by $IC_{80} < 50$ μg/ml		Measured by $IC_{80} < 1$ μg/ml	
Virus clade	Number of viruses	VRC01	CD4-Ig	VRC01	CD4-Ig
A	22	95%	55%	77%	14%
B	49	94%	63%	39%	12%
C	38	82%	68%	37%	8%
D	8	75%	88%	25%	25%
CRF01_AE	18	83%	56%	17%	0%
CRF02_AG	16	75%	75%	38%	6%
G	10	90%	60%	50%	0%
CRF07_BC	11	91%	91%	18%	0%
Other	18	78%	61%	67%	0%
Total	190	86%	66%	42%	8%

*** Data from Wu et al (*S4*)**

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APPENDIX

List of 147 Heavy Chain Sequences

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